

FORM PTO-1390 (REV. 11-2000)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER MPG-108 (24299.08)	
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371				U.S. APPLICATION NO. (If known, see 37 CFR 1.5 Not yet assigned. <b>09/786167</b>	
INTERNATIONAL APPLICATION NO. PCT/EP98/05539		INTERNATIONAL FILING DATE 1 September 1998		PRIORITY DATE CLAIMED 1 September 1998	
TITLE OF INVENTION <b>ELECTRICALLY CONTROLLED TRANSPORT OF CHARGED PENETRANTS ACROSS BARRIERS</b>					
APPLICANT(S) FOR DO/EO/US <b>Gregor Cevc</b>					
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:					
1. <input checked="" type="checkbox"/> This is a <b>FIRST</b> submission of items concerning a filing under 35 U.S.C. 371. 2. <input type="checkbox"/> This is a <b>SECOND</b> or <b>SUBSEQUENT</b> submission of items concerning a filing under 35 U.S.C. 371. 3. <input type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below. 4. <input type="checkbox"/> The US has been elected by the expiration of 19 months from the priority date (Article 31). 5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2)) a. <input checked="" type="checkbox"/> is attached hereto (required only if not communicated by the International Bureau). b. <input type="checkbox"/> has been communicated by the International Bureau. c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US). 6. <input type="checkbox"/> An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)). a. <input type="checkbox"/> is attached hereto. b. <input type="checkbox"/> has been previously submitted under 35 U.S.C. 154(d)(4). 7. <input type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)) a. <input type="checkbox"/> are attached hereto (required only if not communicated by the International Bureau). b. <input type="checkbox"/> have been communicated by the International Bureau. c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired. d. <input type="checkbox"/> have not been made and will not be made. 8. <input type="checkbox"/> An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)). 9. <input checked="" type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). 10. <input type="checkbox"/> An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).					
<b>Items 11 to 20 below concern document(s) or information included:</b>					
11. <input checked="" type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98. 12. <input checked="" type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included. 13. <input checked="" type="checkbox"/> A FIRST preliminary amendment. 14. <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment. 15. <input type="checkbox"/> A substitute specification. 16. <input type="checkbox"/> A change of power of attorney and/or address letter. 17. <input type="checkbox"/> A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825. 18. <input type="checkbox"/> A second copy of the published international application under 35 U.S.C. 154(d)(4). 19. <input type="checkbox"/> A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4). 20. <input type="checkbox"/> Other items or information:					

U.S. APPLICATION NO. (if known) see 7 CFR 1.51 Not yet assigned <b>09/786167</b>		INTERNATIONAL APPLICATION NO. <b>PCT/EP98/05539</b>		ATTORNEY'S DOCKET NUMBER <b>MPG-108 (24299.08)</b>	
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21. <input type="checkbox"/> The following fees are submitted: <b>BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):</b> Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO. .... <b>\$1000.00</b>  International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO ..... <b>\$860.00</b>  International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO ..... <b>\$710.00</b>  International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) ..... <b>\$690.00</b> International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) ..... <b>\$100.00</b> <b>ENTER APPROPRIATE BASIC FEE AMOUNT =</b>				<b>CALCULATIONS PTO USE ONLY</b>	
				\$ 1,000.00	
Surcharge of <b>\$130.00</b> for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				\$	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	\$	
Total claims	39 - 20 =	19	x <b>\$18.00</b>	\$ 342.00	
Independent claims	2 - 3 =	0	x <b>\$80.00</b>	\$	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)				+ <b>\$270.00</b>	\$
<b>TOTAL OF ABOVE CALCULATIONS =</b>				\$	
<input checked="" type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.				+ \$ 1,342.00	
<b>SUBTOTAL =</b>				\$ 671.00	
Processing fee of <b>\$130.00</b> for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				\$	
<b>TOTAL NATIONAL FEE =</b>				\$	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). <b>\$40.00</b> per property +				\$ 40.00	
<b>TOTAL FEES ENCLOSED =</b>				\$ 711.00	
				Amount to be refunded:	\$
				charged:	\$

a. ☒ A check in the amount of \$ 711.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. \_\_\_\_\_ in the amount of \$ \_\_\_\_\_ to cover the above fees.  
 A duplicate copy of this sheet is enclosed.

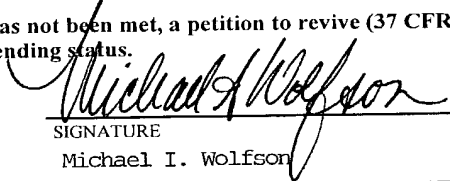
c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any  
 overpayment to Deposit Account No. 03-3415. A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card  
 information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

**NOTE:** Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR  
 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO.

  
 SIGNATURE  
 Michael I. Wolfson  
 NAME  
 24,750  
 REGISTRATION NUMBER

09/786167

JC02 Rec'd PCT/PTO 28 FEB 2001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Gregor Cevc  
For : ELECTRICALLY CONTROLLED TRANSPORT OF CHARGED  
PENETRANTS ACROSS BARRIERS  
Filed : New National Phase Application based on PCT/EP98/05539

February 28, 2001

PRELIMINARY AMENDMENT AND  
INFORMATION DISCLOSURE STATEMENT

Honorable Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

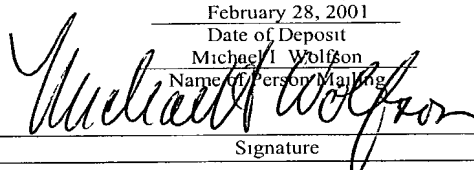
Prior to calculation of the filing fee and examination of the application, please amend the application as follows:

IN THE ABSTRACT

Please add the following Abstract of the Disclosure as a separate page to the application as annexed hereto.

--ABSTRACT OF THE DISCLOSURE

It is an object of the invention to provide a preparation comprising penetrants formed by single molecules or by arrangements of molecules, said penetrants being capable of

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I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. 1.10 on the date indicated above and is addressed to Assistant Commissioner for Patents, Washington, D.C. 20231
February 28, 2001 Date of Deposit
Michael I. Wolfson Name of Person Mailing
 Signature

penetrating the pores of a barrier even when the average diameter of said barrier pores is less than the average diameter of said penetrants, since the penetrants are adaptable to the pores, and said penetrants being capable of transporting agents through said pores after the penetrants have entered said pores; the average diameter and the adaptability of said penetrants being selected, and said penetrants and/or said agents being provided with sufficient electrical charges, to enable and/or control agent transport through said pores by said penetrants, or agent permeation through said pores after penetrant entry into said pores, under the influence of a suitable electrical driving force, said selection at the same time maintaining sufficient penetrant stability. It is another object of the invention to provide a method for effecting the electrically driven transport of said penetrants and associates molecules through the pores in a barrier.--

IN THE CLAIMS

In claim 4, line 1, please delete "any one of claims 1 to 3," and insert --claim 1,--;

In claim 5, line 1, please delete "any one of claims 1 through 4," and insert --claim 1,-  
-;

In claims 6 and 7, line 1 of each claim, please delete "any one of claims 1 through 5," and insert --claim 1,--;

In claim 8, line 1, please delete "claim 6 or 7," and insert --claim 6,--;

In claim 11, line 1, please delete "9 or 10,";

In claim 14, line 1, please delete "any one of claims 8 through 13," and insert --claim  
8,--;

In claims 15 and 16, line 1 of each claim, please delete "any one of claims 8 through  
14," and insert --claim 8,--;

In claim 17, line 1, please delete "any one of claims 8 through 16," and insert --claim  
8,--;

In claims 18 and 19, line 1 of each claim, please delete "any one of claims 8 through 17," and insert --claim 8;--.

In claim 20, line 1, please delete "any one of claims 8 through 19," and insert --claim 8--;.

In claim 21, line 1, please delete "any one of claims 8 through 20," and insert --claim 8,--;

In claim 22, line 1, please delete "any one of claims 8 through 21," and insert --claim 8,--;

In claims 25 and 26, line 1 of both claims, please delete "claims 23 or 24," and insert -  
-claim 23,--;

In claim 27, line 1, please delete "any one of claims 23 through 26," and insert --claim 23,--;

In claim 28, line 1, please delete "any one of claims 23 to 27," and insert --claim 23,  
--;.

In claim 29, line 1, please delete "any one of claims 23 to 28," and insert --claim 23,  
--;

In claim 31, line 1, please delete "claims 29 or 30," and insert --claim 29--;

In claims 32 and 33, line 1 of both claims, please delete "claims 29, 30 or 31," and  
insert --claim 29,--;

In claim 34, line 1, please delete "any one of claims 23 through 33," and insert --claim  
23,--;

In claim 35, line 1, please delete "any one of claims 23 through 34," and insert --claim 23,--;

In claim 36, line 1, please delete "any one of claims 23 through 35," and insert --claim 23,--;

In claim 38, line 1, please delete "claims 36 or 37," and insert --claim 36,--; and

In claim 39, line 1, please delete "any one of claims 23 to 38," and insert --claim 23, --.

## REMARKS

This Preliminary Amendment and Information Disclosure Statement is submitted prior to calculation of the filing fee in order to remove the multiple dependent claims in the PCT application. Upon entry of the Amendment there will be no more multiple dependent claims remaining in the application.

Pursuant to the duty of disclosure set forth in 37 C.F.R. § 1.55(a) and consistent with 37 C.F.R. §§ 1.97 and 1.98, applicant respectfully submits herewith a PTO Form 1449 identifying the citations of which he is aware, which may be material to the examination of this application and in respect of which there may be a duty to disclose in accordance with 37 C.F.R. §§ 1.55 and 1.56.

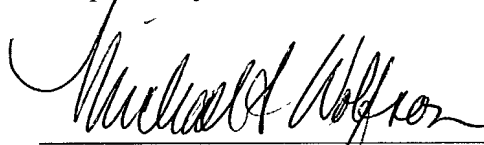
This Information Disclosure Statement is not intended to constitute an admission that any of the patents referred to herein is "prior art" unless specifically designated as such. The filing of this Information Disclosure Statement shall not be construed to mean that a search has been made or that no other material information has been found in 37 C.F.R. § 1.56(a) exists.

The citations identified in the accompanying Form 1449 include those identified in the accompanying International Search Report conducted in the PCT phase and identified by applicant in the Background of the Invention. It is respectfully requested that each of these citations be made of record in connection with the examination of this application. Pursuant to the Tripartite Agreement referenced in MPEP 1893.03(g), copies of the citations should be in the application file. Accordingly, copies are not being forwarded, but should be considered by the Examiner and cited on the face of the patent to issue.

The Examiner is respectfully requested to review the application at an early date with a view towards issuing a favorable action thereon. If upon review of the application, the Examiner is unable to issue an immediate Notice of Allowance, the Examiner is respectfully requested to telephone the undersigned attorney with a view towards resolving the outstanding issues.

Early and favorable action is earnestly solicited.

Respectfully submitted,



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Registration No. 24,750  
Attorney for Applicant  
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12/PR TS

**Electrically controlled transport of charged penetrants**  
**across barriers**

5 **Field of the invention**

This invention relates to a preparation comprising penetrants formed by single molecules or by arrangements of molecules, said penetrants being capable of penetrating the pores of a barrier even when the average diameter of said barrier pores is less than  
10 the average diameter of said penetrants, since the penetrants are adaptable to the pores, and said penetrants being capable of transporting agents through said pores, or enabling agent permeation through said pores after the penetrants have entered said pores; the average diameter and the adaptability of said penetrants being selected, and said penetrants and / or said agents being provided with sufficient electrical charges, to  
15 enable and / or control agent transport through said pores by said penetrants, or agent permeation through said pores after penetrant entry into said pores, under the influence of a suitable electrical driving force, said selection at the same time maintaining sufficient penetrant stability. This invention also relates to a method for effecting the electrically driven transport of said penetrants and associated molecules through the  
20 pores in a barrier.

**Background of the invention**

25 Charged entities may migrate spontaneously from the high to the low electrostatic potential site, unless prevented from doing so by an obstacle, such as a barrier. The driving electrostatic force is proportional to the total charge on an entity and to the electrostatic potential difference. Material flow also depends on the system's resistance to resulting motion. Consequently, the electrically driven transport across a barrier is  
30 sensitive to the number, width and characteristics of pores in a barrier, which together define the barrier permeability,  $P$ , and its inverse, the barrier resistance. One example





The widest channels are negative inside. Neutral channels are only half as wide and the positive ones are twice smaller (Pikal, M.J.; Shah, S. (1990b) Transport mechanisms in iontophoresis. II. Electroosmotic flow and transference number measurements for hairless mouse skin. Pharm. Res. 7: 213-21). To date, the widest channels inferred to occur during a low-voltage electromotion through the skin were reported to be merely 20 nm in diameter or less (Aguillelle, V.; Kontturi, K.; Murtomaeki, L.; Ramirez, P. (1994) Estimation of the pore size and charge density in human cadaver skin. J. Contr. Rel. 32: 249-257).

The standard electrical skin permeability enhancement method (electrophoresis) therefore only can improve the transport of relatively small ( $<2$  nm) charged molecules across the organ. Electrophoresis across the skin, consequently, is feasible for certain polypeptides but is practically useless for the delivery of proteins or other large penetrants (for reviews see refs. Green, P.G.; Hinz, R.S.; Kim, A.; Szoka, F.C. Jr.; Guy, R.H. (1991) Iontophoretic delivery of a series of tripeptides across the skin in vitro. *Pharm. Res. Sep*; 8: 1121-7; Green, P. G.; Flanagan, M.; Shroot, B.; Guy, R. (1993) Iontophoretic drug delivery. In: *Pharmaceutical Skin Penetration Enhancement* (Walters, K. and Hadgraft, J., eds.) Marcel Dekker, New York, 297-319; Heith, M.C.; Williams, P.L.; Jayes, F.L.; Chang, S.K.; Riviere, J.E. (1993) Transdermal iontophoretic peptide delivery: in vitro and in vivo. Studies with luteinizing hormone releasing hormone. *J. Pharm. Sci.* 82: 240-3; Singh, S.; Singh, J. (1993) Transdermal drug delivery by passive diffusion and iontophoresis: a review. *Med. Res. Rev.* 13: 569-621; Singh, J.; Bhatia, K.S. (1996) Topical iontophoretic drug delivery: pathways, principles, factors, and skin irritation. *Med. Res. Rev.* 16: 285-96).

Transcutaneous channels opened by the low voltages and tolerably small currents only cover some 0.005 % of the total treated area, despite their seemingly high number ( $< 3 \times 10^8 \text{ cm}^{-2}$ ) (Pikal, M.J. (1990) Transport mechanisms in iontophoresis. I. A theoretical model for the effect of electroosmotic flow on flux enhancement in transdermal iontophoresis. Pharm. Res. 7: 118-26). More extended local skin perforations, which are created by a higher voltage ( $> 150 \text{ V}$ ), are rarer, but normally

persist in the skin for several days in the form of lesions.

- Transcutaneous channels size is affected by a number of parameters. For example, a channel widens with increasing electrostatic potential as well as with decreasing supporting electrolyte concentration, but the latter variation is practically only possible within relatively narrow limits. Moreover, no teaching was given to date on how to improve the transport across a barrier by using such principles. Perhaps, this is due to the fact that the electrophoretic channels in the skin are charge and molecular weight selective (Banga, A.K.; Chien, Y.W. (1993) Hydrogel-based iontotherapeutic delivery devices for transdermal delivery of peptide/protein drugs. *Pharm. Res.* 10: 697-702) but not very sensitive to the agent lipophilicity variation (Green, P.G.; Hinz, R.S.; Kim, A.; Szoka, F.C. Jr.; Guy, R.H. (1991) Iontophoretic delivery of a series of tripeptides across the skin in vitro. *Pharm. Res.* Sep; 8: 1121-7).
- Repeated electrophoretic delivery through the same skin area results in a divergent, but typically greater, flux across the barrier which makes data interpretation and recommendations difficult (Heith, M.C.; Williams, P.L.; Jayes, F.L.; Chang, S.K.; Riviere, J.E. (1993) Transdermal iontophoretic peptide delivery: in vitro and in vivo. Studies with luteinizing hormone releasing hormone. *J. Pharm. Sci.* 82: 240-3). Further complications arise from the electrical current through the appendages in the skin, such as hair follicles.

Electrical opening of channels through the skin is reflected in the following contribution to the skin permeability,

$$P_{i,e} = \text{Skin Permeability to Ions} = (c_i Z_i F / RT) D_i / d_s$$

which needs to be added to the permeability observed with no electrical force applied across the barrier. In addition to the pores opening, a transcutaneous electrical potential gradient ( $\Delta\psi_{el}$ ) also activates the electromotive forces which try to drive charged penetrants through the channels. This gives rise to an additional term in Fick's transport

equation used to model transbarrier (e.g. transcutaneous) transport (see further discussion):

$$j_i = \dots a c_i + P_{i,el} \Delta\psi_{el}$$

5

$c_i$  is the bulk concentration of substance  $i$ ,  $Z_i F$  is its molar charge (valency times Faraday constant), and  $RT$  is the molar thermal energy.  $D_i$  is the diffusivity of substance  $i$  and  $d_s$  the skin thickness. Electric current corresponding to  $i$  is given by the product of  $i$ -flux and  $Z_i F$ , but the total current comprises all individual contributions and thus is given by the sum of such contributions.

10

Electrophoresis represents the direct flow of charged molecules in an electric field under the electrode. Drug molecules must therefore be placed at electrodes having a polarity of the same charge as the agent. Under such circumstances, the flux magnitude is proportional to the net number of charges on each migrating molecule and to the applied potential. Further important factors are drug concentration and diffusivity in the barrier or skin (see equation given later in the text).

15

Owing to absolute differences in concentrations, electrophoretic current normally also comprises contributions from the supporting electrolyte ions (e.g.  $\text{Na}^+$ ,  $\text{Cl}^-$ ). These are often dominant making the drug contribution only a minor part of the measured current. Increased ion concentration under the electrodes therefore lowers the useful part of electrophoretic flow (Pikal, M.J.; Shah, S. (1990b) Transport mechanisms in iontophoresis. II. Electroosmotic flow and transference number measurements for hairless mouse skin. Pharm. Res. 7: 213-21; Pikal, M.J.; Shah, S. (1990c) Transport mechanisms in iontophoresis. III. An experimental study of the contributions of electroosmotic flow and permeability change in transport of low and high molecular weight solutes. Pharm. Res. 7: 222-9), as can be seen from simple differential calculation.

25

It is therefore state of the art knowledge that physical limitations restrict maximum achievable, or tolerable, electrophoretic current across the skin: currents flowing

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through the already opened pores dissipate electric energy; this prevents a greater increase in the channel number and size, as stated above, and minimizes the achievable transport gain. Further pore opening is also restricted by the adverse side effects of energy dissipation in the skin (skin itching and etching).

5

Electro-osmotic flux, that is, the flow of water associated with the transported ions carrying uncharged species, also relies on hydrophilic channels in the skin and on the applied potential. Flux under an anode typically exceeds the cathodal values, probably due to the different average size of positively and negatively charged channels. The average flux value reaches a steady-state during 10 hours of constant-current iontophoresis ( $0.36 \text{ mA cm}^{-2}$ ) at the level a few times higher than at the beginning ( $< 3 \mu\text{L h}^{-1} \text{ cm}^{-2}$ ; Kim, A.; Green, P.G.; Rao, G.; Guy, R.H. (1993) Convective solvent flow across the skin during iontophoresis. Pharm. Res. 10: 1315-20).

15 Electrically induced changes in the skin are the greatest over the first hour of electrophoresis (Pikal, M.J.; Shah, S. (1990b) Transport mechanisms in iontophoresis. II. Electroosmotic flow and transference number measurements for hairless mouse skin. Pharm. Res. 7: 213-21; Craane van-Hinsberg, W.H.; Bax, L.; Flinterman, N.H.; Verhoef, J.; Junginger, H.E.; Bodde, H.E. (1994) Iontophoresis of a model peptide across human skin in vitro: effects of iontophoresis protocol, pH, and ionic strength on peptide flux and skin impedance. Pharm. Res. Sep; 11: 1296-300). During this period of time, the resistance drops from  $> 20 \text{ k}\Omega \text{ cm}^{-2}$  to approximately 10 % of the starting value.

25 Skin pretreatment with ethanol reduces (Brand, R.M.; Iversen, P.L. (1996) Iontophoretic delivery of a telomeric oligonucleotide. Pharm. Res. 13: 851-4) or else increase (Srinivasan, V.; Higuchi, W.I.; Sims, S.M.; Ghanem, A.H.; Behl, C.R. (1989) Transdermal iontophoretic drug delivery: mechanistic analysis and application to polypeptide delivery. J. Pharm. Sci. 78: 370-5) the electrophoretic transport across the organ. Most chemical skin permeation enhancers improve the electroconductivity of the skin, and thus also the electrically driven transcutaneous transport (Green, P.G.; Hinz,

30



of the desire to deliver transcutaneously large molecules, such as peptides and proteins, but also due to the long standing desire to control aggregate motion across any kind of transport barrier.

- 5 The extent and mechanism of aggregate motion across biological barriers, such as the stratum corneum, is strongly disputed (Cevc, G. (1996) Lipid Suspensions on the Skin. Permeation Enhancement, Vesicle Penetration and Transdermal Drug Delivery. Crit. Rev. Therap. Drug Carrier Systems. 13: 257-388), as is the penetration pathway through the skin. We have repeatedly discussed hydrotaxis as most important cause for the
- 10 transport of superficially hydrophilic, highly deformable vesicles through the biological barriers, such as the skin (Cevc, G. (1996) Lipid Suspensions on the Skin. Permeation Enhancement, Vesicle Penetration and Transdermal Drug Delivery. Crit. Rev. Therap. Drug Carrier Systems. 13: 257-388; Cevc, G. (1997) Drug Delivery Across the Skin. Exp. Opin. Invest. Drugs 6: 1887-1937). We argued that diffusion is not a good basis
- 15 for transporting large aggregates, i.e. lipid vesicles, accross such barriers.

The first reason for this is the very low permeability ( $P_a$ ) of any big aggregate with a large effective mass, which is typically proportional to the aggregation number ( $n_a$ ). Since the  $P_a$ -value correlates with the diffusion constant ( $D_a$ ), the permeability and the

- 20 flux of such a large body both decrease linearly with the growing aggregate size. ( $P_a$  is thus proportional to  $D_a \sim D_1/n_a$ , where  $D_1$  is the diffusion constant of a monomer.)

The second cause for the insignificance of aggregate diffusion is the smallness of largest achievable aggregate concentration difference across the barrier ( $\Delta c_a = \Delta c_1/n_a$ , where  $c_1$

- 25 is the saturated monomer concentration). As can be calculated from the first Fick's law, the flux  $j_m = P_a \Delta c_a$ , is hence proportional to  $D_a \Delta c_a \sim D_1 \Delta c_1/n_a^2$ .

Both above mentioned phenomena, which result in  $D_a(n_a \gg 1) \rightarrow 0$  and  $\Delta c_a(n_a \gg 1) \rightarrow 0$ , contribute to negligibly small barrier permeability for the vesicle

- 30 transport:  $P_a(n_a \gg 1) \rightarrow 0$ .

The use of water activity gradient ( $\Delta a_w$ ), i.e. hydrotaxis, to drive transbarrier transport solves the first part of permeability problem. Our interpretation of this is the following: the aggregate independent water activity gradient exerts a similar attraction on all polar molecules in the aggregate; this strengthens proportionally the pressure acting on each aggregate,  $\Delta p_{hyd,a} \sim \Delta a_w RT n_a$ , or on the corresponding force that drives the aggregate transport across the barrier,  $F_{hyd}$ . Both are much bigger for aggregates than for a single molecule. This compensates the smallness of aggregate concentration difference, as can be seen from the generalized Fick's equation:

$$j_a = P_a \Delta c_a + P_a'' \Delta a_w RT n_a \\ \sim P_a' n_a F_{hyd,l}$$

$F_{hyd,l}$  denotes the force acting on each monomer in the aggregate and  $RT$  is the thermal energy.

In order to profit maximally from an 'external' transport driving force, which is (permeant/penetrant) concentration independent, one can use easily deformable aggregates described in PCT/EP91/01596. This minimizes the increase of transport resistance with increasing aggregate number, by pushing it below the suggested linear dependence (see figure 1).

One example for this are the vesicles with a membrane sufficiently flexible to result in a low vesicle deformation energy. This is especially true when the vesicles are subject to strongly anisotropic (ideally: unidirectional) stress, 'force' or pressure. The combination of molecular aggregation and membrane flexibility under the corresponding conditions, therefore, may lead to vesicle motion through a barrier even when the pores in such barrier are smaller than the vesicle diameter. Significant material flow in the desired direction can result from this.

The same consideration applies to every external, and therefore concentration



independent, force ( $F_{ext}$ ) or pressure ( $\Delta p_{ext}$ ). The proviso for this is that aggregate-size dependent increase of the force - or of the resulting trans-barrier pressure difference - exceeds the decrease of permeability ( $P_a$ ) with growing aggregate size. A net trans-barrier transport results from this. The relation is schematically illustrated in figure 1.

- 5 If the driving force increases linearly with the number of charges on each moving entity, and provided that the transport resistance of such entity is increases less rapidly than the driving force (full line), a net transport will ultimately result. Such is the situation with the penetrants which are adaptable in shape to the pores. When the penetrants get big enough, and the driving force exceeds the transport resistance, an effective transport sets in. If the penetrants are not adaptable to the pores in a barrier, however, the barrier resistance inevitably exceeds the driving force, when the average 'penetrant' size exceeds the average diameter of a pore (dotted line).
- 10

The above reasoning applies as long as the transport driving force is constant or as long as the increase of transport resistance does not exceed the size dependent ascent of driving force. Highly deformable aggregates subject to a sufficiently high external pressure ( $\Delta p_{ext,a}$ ) provide an example for this; the opposite situation is encountered with conventional, less deformable aggregates under a similar pressure, since these aggregates will rather break at than pass through the barrier.

15

20 In the case of an external electrical force ( $F_{el}$ ), which could lead to so-called electrophoresis, similar basic principles apply, but are more complicated and not a priori recognizable..

- 25 In the simplest, hypothetical case, in which an aggregate comprises  $n_a$  charged molecules with a charge  $Ze_0$  each, and the corresponding counterions are the only other charged entities in the system, the rate of aggregate transport increases linearly with aggregate size or number, as well as with the electric gradient across the barrier (E). This is true as long as transport resistance remains constant, as the driving force is then given by  $F_{el} = n_a Ze_0 E$ , and the  $P_a$ -value is taken to be constant. However, if the transport resistance depends on aggregate-size, and also increases with the value of  $n_a$ ,
- 30





It was therefore not expected to date that electrical driving force could be used as a replacement of hydrotaxis for the purpose of transporting large lipid aggregates (e.g. vesicles) or other big entities across the skin.

- 5 In view of the foregoing it is therefore an object of the present invention to provide a preparation comprising penetrants formed by single molecules or by arrangements of molecules, said penetrants being capable of penetrating the pores of a barrier even when the average diameter of said barrier pores is less than the average diameter of said penetrants, since the penetrants are adaptable to the pores, and said penetrants being
- 10 capable of transporting agents through said pores, or enabling agent permeation through said pores after the penetrants have entered said pores.

- It is moreover an object of the present invention to provide a method for effecting the electrically driven transport of penetrants and associated molecules through the pores in
- 15 a barrier by applying an electrical potential across the barrier.

These objects are attained by the invention as defined in the attached independent claims.

- 20 Further advantageous embodiments of the present invention are provided by appended subclaims.

### **Description of the invention**

- 25 We found, unexpectedly, that charged ultradeformable lipid aggregates in vesicular form can be forced to cross artificial as well as natural nano-porous barriers with an externally applied electrical potential difference. The proviso for this is sufficient stress on the vesicles, which must be big enough to deform the vesicles. Such a situation is only realized with the vesicles with very flexible membranes. The process involving the
- 30 deformation of entire aggregate, the relative magnitude of vesicle adaptation to the pore

penetration is affected by the average vesicle/pore size ratio.

We also found out that the electromotion of charged aggregates is sensitive to the bulk electrolyte concentration. Unexpectedly, the measured dependence was seen to deviate, qualitatively as well as quantitatively, from that expected on the basis of known electromotion of non-deformable, small penetrants across a barrier. The electrically driven motion of the highly deformable vesicles across "confining" pores therefore differs from conventional electrophoresis and provides new means for the delivery of drugs across various, including biological, barriers.

10

It stands to reason that nonocclusive pretreatment of the skin with a suspension of ultradeformable aggregates - and subsequent application of transcutaneous electric potential - can increase the final electrophoretic flux. We speculate that this might be due to the opening of channels in the skin by non-electrical means. Such an increase in overall penetrability of the organ, which relies on more efficient, non-electrostatic channels opening, can be exploited subsequently to deliver loaded charged vesicles across the barrier. The latter are then pushed through pretreated skin by the transcutaneous electric potential applied under occlusion.

20 Last but not least, it is also plausible to postulate that, contrary to previous belief, large molecules can be delivered efficiently across the skin. Their delivery is made possible after macromolecular association with the charged ultradeformable carriers and involves transcutaneous electromotion of such unusually adaptable, protein-carrying transporters.

25 We further discuss some relevant properties of molecular aggregates/associates suitable for the use in conjunction with electrophoresis. We concentrate only on the complex bodies that can overcome transport barriers under the influence of a transbarrier electrical gradient of sufficient magnitude. We describe the basic experiments relevant for this phenomenon and interpret their results. We propose general conclusions useful for the application of concepts advocated in this work in the widest possible sense. 30 Particularly interesting, but not exclusive, is the use of our novel approach in the human

and veterinary medicine.

In the present invention, permeation denotes diffusive, concentration driven motion of molecules across a barrier. Penetration describes the non-diffusive motion of large  
5 penetrants across a barrier; the process typically being associated with a penetration induced decrease in the barrier resistance (pore widening or channel opening).

A penetrant, consequently, is any entity comprising a single molecule or an arrangements of molecules too big to permeate through the barrier. A permeant, on the  
10 other hand, is an entity that can permeate through the (semi-permeable) barrier. A penetrant in an external field experiences driving force proportional to the nominal penetrant size and to the applied field. Such a force may push the penetrant through the barrier, such as the skin, if the force is strong enough either to deform the penetrant or else to widen the passages in the barrier sufficiently to elude the problem of size  
15 exclusion, or both. In the skin, for example, a transport-driving force must first intercalate the penetrant between cells to form channel wider than the effective penetrant size. (To achieve a high rate of penetrant transport, the effective penetrant size should be much smaller than the nominal penetrant size.) This goal is best achieved by the penetrants that are controllably and stress-dependently deformable. The average  
20 diameter, the electrical charge and/or the adaptability in shape or size to the pores of the penetrant is selected so as to enable electromotion.

Electrical potential gradient (across a barrier) means an arbitrary potential difference of any sign or magnitude, unless otherwise specified. Specifically, it is not necessary to  
25 place the potential generating electrodes directly on the barrier; any placement resulting in trans-barrier gradient is acceptable. "Potential difference" is used as a synonym for "potential gradient".

For further definitions, especially such pertaining to the highly deformable complex  
30 bodies (aggregates) and their mechanism of action, as well as for the list of selected interesting agents, we explicitly refer to our issued or pending patents (DE 41 07 152.



temperatures are also possible. They make particular sense with the formulations comprising from synthetic substances which are rigid between the room and skin or other barrier temperature.

5 Formulations for the use in conjunction with electrophoresis can be processed at the site of application. For lipid vesicles, both charged and uncharged, examples are given in our previous german patent application and in the handbook on 'Liposomes' (Gregoriadis, G., Hrsg., CRC Press, Boca Raton, Fl., Vols 1-3, 1987), in the monography 'Liposomes as drug carriers' (Gregoriadis, G., Hrsg., John Wiley & Sons,  
10 New York, 1988), or in the laboratory manual 'Liposomes. A Practical Approach' (New, R., Oxford-Press, 1989). If required, any suspension of drugs can also be diluted or concentrated (e.g. by ultracentrifugation or ultrafiltration) just before application; additives can also be given into a formulation at this time or before. After any system manipulation, the carrier characteristics should be checked and, if required, readjusted.

15

This invention concerns a preparation comprising penetrants formed by single molecules or by arrangements of molecules, in which said penetrants are capable of penetrating the pores of a barrier even when the average diameter of said barrier pores is less than the average diameter of said penetrants, since the penetrants are adaptable to  
20 the pores. In this the penetrants are capable of transporting agents through the pores in the barrier. When the penetrants enter the pores a pore widening and channel opening by the penetrants results. Therefore, alternatively, agent permeation through the (now opened or widened) pores in the barrier is enabled subsequently to penetrant entry into said pores. The average diameter and the adaptability of said penetrants are selected, and  
25 said penetrants and / or said agents are provided with sufficient electrical charges, to enable and / or control agent transport through said pores by said penetrants, or in the alternative case agent permeation through said pores after penetrant entry into said pores, under the influence of a suitable electrical driving force, said selection at the same time maintaining sufficient penetrant stability

30

According to the invention it is preferred if said penetrant is provided with sufficient



electrical charges, at least when associated with an agent, and the penetrant could, in the absence of an electrical driving force, not readily penetrate the barrier pores; the average diameter, the kind and amount of electrical charges and / or the adaptability of the electrically charged penetrants or the charged associations of penetrant and agent, being  
5 selected to achieve, and in case. control said transport through the barrier under the influence of the electrical driving force.

It further is preferred if said penetrant is provided with sufficient electrical charges, at least when associated with an agent, and the penetrant could penetrate the barrier pores  
10 in the absence of an electrical driving force; the average diameter, kind and amount of electrical charges and / or the adaptability of the electrically charged penetrants or the charged associations of penetrant and agent being selected to provide control of the agent transport through the barrier under the influence of an electrical driving force

15 Furthermore it is preferred if said penetrant is capable of penetrating said pores under the influence of a suitable driving force, which may be an electrical driving force when the penetrant is suitably electrically charged, and the agents being sufficient electrically charged to enable and / or control their permeation through the pores of the barrier subsequent to entry of said penetrant into said pores.

20

Said electrically charged penetrants or the charged association of penetrant and agent have preferably an average diameter which is greater (by at least the factor of 2) than the average diameter of the pores of the barrier.

25 In a particular embodiment of the invention a preparation is described which is characterized by the fact that the electrically charged penetrant is formed by an electrically charged single molecule or an arrangement of electrically charged molecules and is associated with one or several charged or uncharged agent molecules.

30 As a variation the above mentioned penetrant is formed by an electrically neutral single molecule or an arrangement of electrically neutral molecules and is associated with at

least one electrically charged agent, the quantity of electrical charges being sufficient to enable transport.

In a preferred embodiment of the invention said penetrants are suspended or dispersed  
5 in a liquid medium and comprise arrangements of molecules in the form of minute fluid droplets surrounded by a membrane-like coating of one or several layers of at least two kinds or forms of amphiphilic substances with a tendency to aggregate, said at least two substances differing by at least a factor of 10 in solubility in the, preferably aqueous, liquid medium, such that the average diameter of homo-aggregates of the more soluble  
10 substance or the average diameter of hetero-aggregates comprising both said substances is smaller than the average diameter of homo-aggregates of the less soluble substance.

It turns out to be advantageous if the more soluble substance is the agent to be transported through the barrier, and has a propensity to form common larger structures  
15 with the less soluble substance. The common structure may comprise a physical or chemical complex of the substances.

According to the invention it is convenient if the more soluble substance tends to solubilize the penetrant droplet and the content of this substance is up to 99 mol% of the  
20 concentration required to solubilize the droplet, or else corresponds to up to 99mol% of the saturating concentration in the unsolubilized droplet, whichever is higher.

It is preferred if the content of the more soluble substance is below 50 %, especially below 40 % and most preferably below 30 %, of the respective solubilizing  
25 concentration of said substance.

According to the invention the content of the more soluble substance is below 99 %, preferably below 80 % and most preferably below 60 % of the saturation concentration of said substance in the droplet.  
30

It is advantageous if the less soluble self-aggregating substance is a lipid-like substance

and the more soluble substance is a surfactant.

It is preferred if the average diameter of the penetrant is between 40 nm and 500 nm, preferably between 50 nm and 250 nm, even more preferably between 55 nm and 150 nm and particularly preferably between 60 nm and 120 nm.

It is also preferred if the average diameter of the penetrant is 2 to 25 times bigger than the average diameter of the pores in the barrier, preferably between 2.25 and 15 times bigger, even more preferably between 2.5 and 8 times bigger and most preferably between 3 and 6 times bigger than said average pore diameter.

According to the invention it is further advantageous if the average net surface charge density on a droplet is between  $0.05 \text{ Cb m}^{-2}$  (Coulomb per square meter) and  $0.5 \text{ Cb m}^{-2}$ , preferably between  $0.075 \text{ Cb m}^{-2}$  and  $0.4 \text{ Cb m}^{-2}$ , and particularly preferably between  $0.10 \text{ Cb m}^{-2}$  and  $0.35 \text{ Cb m}^{-2}$ .

It is preferred if the weight amount of droplets in formulations for use on human or animal skin is 0.01 to 40 weight-% of the total preparation mass, in particular between 0.1 and 30 weight-%, and particularly preferably between 5 and 20 weight-%.

It is also preferred if the weight amount of droplets in formulations for the use on human or animal mucosa is 0,0001 to 30 weight-%.

Specific embodiments of the invention are disclosed in which the agent is an adrenocorticostaticum, an adrenolyticum, an androgen or antiandrogen, an antiparasiticum, an anabolicum, an anaestheticum or analgesicum, an analepticum, an antiallergicum, antiarrhythmicum, antiarteroscleroticum, antiasthmaticum and/or bronchospasmolyticum, an antibioticum, antidrepressivum and/or antipsychoticum, an antidiabeticum, an antidot, antiemeticum, antiepilepticum, antifibrinolyticum, anticonvulsivum or anticholinergicum, an enzyme, coenzyme or a corresponding enzyme inhibitor, an antihistaminicum, antihypertonicum, an antihypotonicum,

anticoagulant, antimycoticum, antimyasthenicum, an agent against Morbus Alzheimer or Parkinson, an antiphlogisticum, antipyreticum, antirheumaticum, antisepticum, a respiratory analepticum or a respiratory stimulant, a broncholyticum, cardi tonicum, chemotherapeuticum, a coronary dilatator, a cytostaticum, a diureticum, a ganglium-  
 5 blocker, a glucocorticoid, an antinflue agent, a haemostaticum, a hypnoticum, an immunoglobuline or its fragment or any other immunologically active substance such as an immunomodulator, a cytokine, etc., a bioactive carbohydrate(derivative), a contraceptive, an anti-migraine agent, a corticosteroid, a muscle relaxant, a narcoticum, a neurotherapeutic agent, a (poly)nucleotide, a neurolepticum, a neurotransmitter, a  
 10 (poly)peptide(derivative), an opiate, an ophthalmicum, a (para)-sympaticomimeticum or (para)sympathicolyticum, a protein(derivative), a psoriasis/neurodermitis drug, a mydriaticum, a psychostimulant, a rhinologicum, a sleep-inducing agent, a sedating agent, a spasmolyticum, tuberlostaticum, urologicum, a vasoconstrictor or vasodilatator, a virustaticum, a wound-healing substance, an inhibitor (antagonist) or promoter  
 15 (agonist) for the activity of any of the above-mentioned agents or any combination of such agents.

It is particularly advantageous if the liquid medium characteristics, especially the concentration and the composition of the supporting electrolyte, are selected so as to  
 20 enable and / or control the rate or the efficiency of transport of the penetrant through the pores of the barrier.

According to the invention the supporting electrolyte, in particular a buffer, is selected among monovalent (1:1) or other low valency electrolytes, with the bulk concentration  
 25 preferably below 150 mM, more preferably below 100 mM, even more preferably below 50 mM, and particularly preferably up to 10 mM.

Further, according to the invention a method for effecting the electrically driven transport of said penetrants and associated molecules through the pores in a barrier, as  
 30 above defined, is provided which is characterized by the fact that a sufficient electrical potential is applied across the barrier.

According to the invention the electrodes used to generate the electrical potential across the barrier are located on opposite sides or on the same side of the barrier and are arranged so as to ensure that most of the resulting electrical current will flow across the barrier.

5

It is preferred if the applied electrical potential value is chosen to be below 30 V, more often below 15 V, and even more preferably below 10 V, per  $\text{cm}^2$  of the barrier surface. It is advantageous if the current driven across the barrier by the applied electrical potential is in the physiologically tolerable range, typically below  $2 \text{ mA cm}^{-2}$ ,  
10 preferably below  $1 \text{ mA cm}^{-2}$ , more preferably below  $0.6 \text{ mA cm}^{-2}$  and most preferably up to  $0.4 \text{ mA cm}^{-2}$ .

15

Further it is preferred if the electrode size is less than  $200 \text{ cm}^2$ , more preferably below  $100 \text{ cm}^2$ , especially below  $50 \text{ cm}^2$ , most preferably below  $10 \text{ cm}^2$ , or even below  $5 \text{ cm}^2$ .

20

According to the invention the electrically conductive material on or of the electrodes comprises at least one metal, in particular selected from precious metals, such as silver or palladium, and/or biocompatible salts or chemical complexes of such metals, preferably the biocompatible chlorides, and most preferably silver chloride.

25

It is advantageous if at least one the electrode compartment is loaded with electrically charged penetrants.

It has also been shown that it is advantageous if the electrode is loaded at the application site or earlier.

It then is preferred if the electrode is loaded shortly before application, preferably within 360 min, more preferably within 60 min and even more preferably within 30 min.

30

In a preferred embodiment of the invention the electrode is loaded with the electrically charged penetrant pre-associated with molecules to be transported, in particular

(biologically active) agents.

In another preferred embodiment of the invention the electrode is loaded with the penetrant and the molecules to be transported, in particular agents, that associate  
5 therewith during or after said loading.

In preferred embodiments of the invention one or more programmable, preferably small, hand-held or self-supported, for example wrist-watch like, devices for single or repeated use are employed to control the polarity, magnitude and / or time-dependence of applied  
10 electric potential.

It is advantageous if different treatment areas are selected to control the transport.

In another preferred embodiment of the invention the barrier is pretreated before  
15 initiating the electrically driven transport of charged penetrants, by a non-occlusive application of suitable penetrants on the modifiable barrier, especially formed by human or animal skin, to increase the number or width of penetratable pores in the barrier subsequently to be used for the electrically driven transport across said pre-treated skin barrier.

20

It is preferred if the charged or uncharged penetrants used to pre-treat the barrier are similar or identical with those employed for the subsequent electrically driven transport.

It is advantageous if the charged or uncharged penetrants are non-occlusively applied for  
25 up to 24 hours or even longer, typically for up to 12 hours, especially up to 3 hours, or more preferably for less than 1.5 hours, and in case even for less than 30 min, prior to the initiation of electrically driven transport of charged penetrants and/or permeants across the barrier. It shall be emphasized that it is a characteristic feature of the present invention that the electrically driven transport of permeants, i.e. any entity being capable  
30 to permeate through the pores in the barrier, may be enhanced by a pre-treatment of the barrier as above described before initiating the electrically driven transport of the

permeant.

It further is advantageous if the transportation rate, i.e. the flux, of charged penetrants through the barrier pores is determined as a function of the applied electrical potential or of the electrical current across the barrier, and the function thus found is then employed to optimize the preparation or application.

Hereinafter, several illustrative examples of the invention's systems and methods are given; it will be understood that these neither define nor imply limits of this invention. All temperatures are in degree Celsius, carrier sizes are in nanometers, ratios and percentages are given in molar units, unless stated otherwise. Standard SI units are used otherwise in the text.

## EXAMPLES

### General experimental setup and sample preparation

Highly adaptable charged aggregates in the majority of cases studied in this work comprised anionic dioleoylphosphatidylglycerol (DOPG). Additional lipids with detergent or surfactant-like properties (typically the non-ionic Tween 80) were incorporated into lipid bilayers to increase the membrane flexibility. Increasing surfactant-to-lipid ratio made the vesicular aggregates more and more deformable, up to the concentration at which membrane stability was negatively affected by the detergent.

The total lipid concentration was typically 5 w-% and typically diluted to 0.5 %, unless stated otherwise. The bulk phase included buffering ingredients (10 mM) as well as, in some cases, dilute electrolyte (NaCl).

Laboratory made platinum electrodes were used in commercial glass-holders (Crown

Glass, Inc, USA), fixed to the device with a metal clamp. Hard-plastic covers provided with two openings for filling and sampling were pressed tight (sealed with O-rings) on the holder. During experiments, one of these openings was always open to let gas produced by water hydrolysis escape. This should, and has, prevented the bursting of the membrane/holder arrangement.

Freshly cleaned electrodes were separated from the receiving fluid with a microporous membrane (10 nm on the blank side and, for example, 30 nm on the test side). On the donor side, the filling volume was substantially bigger (1.2 mL) than on the blank side (14.5 mL), where the electrode was kept as close to the barrier as possible. The holder was used in a horizontal position to permit stirring of the receiver fluid. Stirring was achieved with a small magnetic bar that revolved on top of the tested barrier. The microporous barrier served as a surrogate or "artificial" skin, for the purposes of this study.

Electrical boundary conditions were defined and maintained with a constant current source (Phoresor: Iomed, Salt Lake City, USA; typical error: 0.1 mA) or a constant voltage source (Siemens, Munich, Germany; typical error: 1 mV). The test suspension was in contact with the cathode, whereas the blank sample volume was contacted by the anode.

The receiving fluid contained charged polymers (alginate acid: 0,25 w-%). These buffering polymers were first dissolved in salt-free water from an Elgastat purification unit (ELGA, UK) and then adjusted to the desired pH range (between 7 and 7.3) by titration with 0.01 N sodium hydroxide. To avoid changes in the mixed lipid vesicles composition, the fluid in the receiver compartment also contained  $10^{-5}$  M of the most soluble vesicle component, that is, the critical micelle concentration of Tween 80. Benzyl alcohol (0.5 volume %) was added to prevent microbial system contamination during the experiments. The receiver fluid was forced by a peristaltic pump to circulate through the cuvette (placed in a fluorimeter) and to pass through the sampling cell into which a pH electrode was inserted. All experiments reported here were run at 37 degrees



Celsius.

Readings were taken continuously. The data were transformed into an electronically  
analysable and storable file using a XT-IBM micro-computer, equipped with an AD-  
5 converter and our own dedicated software.

For the data comparison we focused on the starting period, during which the electrical  
boundary conditions changed by a few percent only. The changes were estimated as  
good as possible and used to assign the errors shown in some figures.

10

To avoid false positive results, the completeness of label-aggregate association was  
confirmed early during the experimental work. To determine the relative amount of  
surfactant-solubilized label in the small mixed lipid micelles or in other kind of  
complexes, various suspensions were tested. This was done by pushing suspension  
15 through the membranes with 10 nm pores. Resulting fluxes were typically very small  
for the DPH labelled suspension. The corresponding flux values, nevertheless, were  
subtracted from the final flux of vesicles (that do not cross 10 nm pores). In experiments  
with epidermis, Rho-DHPE was used as the fluorescent label. Rho-DHPE is still highly  
lipophilic, but more soluble than DPH. Background signals with the former label were  
20 therefore higher than in the case of DPH-labelled vesicles and are shown in the figures  
directly rather than after subtraction from the other data.

### Examples 1-2:

#### Aggregate charge effect

Uncharged highly deformable vesicles:

274 mg phosphatidylcholine (SPC)

226 mg Tween 80 (Tw80)

0.1 mol-% DPH (relative to SPC)

99.5 mL phosphate buffer, 10 mM, pH 7 - 7.3

Vesicle/pore size ratio: 3.3

Charged highly deformable vesicles:

274 mg phosphatidylglycerol (DOPG<sup>-</sup>, as above)

226 mg Tween 80

0.1 mol-% DPH (relative to SPG)

99 mL phosphate buffer, 10 mM, pH 7

Vesicle/pore size ratio: 3.7

Electrical current: 1.2 mA (current density: 0.279 mA/cm<sup>2</sup>)

**Preparation of test suspension.** The lipid mixture was suspended in dilute electrolyte. A sterile glass container containing crude lipid suspension was covered tightly and stirred magnetically for 3 days at room temperature. To narrow down the vesicle size distribution, the suspension was sequentially extruded through polycarbonate membranes of Nucleopore type with a nominal pore size of 400 nm, 100 nm and 50 nm, respectively. This was done at least 20 times. Vesicle suspension was then frozen and thawed 5 times at -70°C and + 50°C, respectively. To get the desired final vesicle size, suspension was re-extruded, 4 times through a 100 nm filter at 0.7 MPa. Finally, the suspension of highly deformable vesicles was sterilized by filtration through a sterile filter with 200 nm pores (Millipore) and stored at 4 °C.

**Electrophoretic measurements.** First, the background diffusion of label molecules  
5 was determined. This was done for several hours without applying an electrostatic  
potential. Next, constant electric current was set and maintained across the barrier.  
During this second period, pH in various parts of the test system was monitored. In  
receiver compartment a digital pH meter was used whereas in donor compartment dip-  
sticks were employed. Electrical potential difference across the barrier was permanently  
10 assessed and recorded as well. Concurrently, the electrical barrier resistance was  
calculated (from the measured potential and current data using Ohm's law).

Fluorescence increase in the flow-through cuvette was monitored continuously.

Fluorescence increase was identified with the transported amount of material. This was done by using results from separate calibration measurements, during which known amounts of labelled suspensions were added directly into the receiving compartment.

- 5 The flow of lipophilic fluorescent label (DPH) across the barrier is believed to be representative of the electrically driven vesicles motion through the barrier. The transport data given in figure 1, consequently, correspond to cumulative effect of vesicle penetration through the barrier. The measured data reveal dramatic differences in the transport of charged and uncharged mixed lipid vesicles through "confining" pores
- 10 in the barrier. This clearly demonstrates the electromotive nature of aggregate transfer discovered and explored in this work.

The lack of noncharged vesicle transport notwithstanding, the applied transbarrier potential does drive a flux of small charged molecules (chiefly ions) across the barrier,

15 as seen from the maintainance of constant current condition.

**Figure 2:** Time dependence of material and vesicle transport across a barrier with an applied electrical potential difference of 1.2 V, which gives rise to the trans-barrier electrical current of 0,279 mA cm<sup>-2</sup>. Charged and uncharged,

20 zwitterionic, lipid vesicles were tested.

### Examples 3-4:

#### Aggregate deformability effect

Conventional charged vesicles, liposomes:

- 500 mg phosphatidylglycerol (DOPG)
- (prepared from from soy-bean phosphatidylcholine)
- 0.1 mol-% DPH (relative to DOPG)
- 99.5 mL phosphate buffer, 10 mM, pH 7

Vesicle/pore size ratio: 2.9

Highly deformable charged vesicles:

274 mg phosphatidylglycerol (DOPG, as above)

226 mg Tween 80

0.1 mol-% DPH

99 mL phosphate buffer, 10 mM, pH 7

Vesicle/pore size ratio: 3.5

Electrical current: 1.6 mA (current density:  $0.381 \text{ mA cm}^{-2}$ )

**Results** obtained with conventional vesicles differ completely from the data measured with highly deformable vesicles: simple charged liposomes do not cross 30 nm pores in the barrier under the influence of an electrical (or, in fact, any other) driving force. The fact that no significant motion of the labelled molecules across the barrier is detected for at least 6 hours supports this conclusion. Conversely, the vesicles with a highly flexible and deformable, and thus better adaptable, membrane tend to move through the narrow pores in a barrier, when they are driven in the right direction by sufficiently strong transbarrier electrical potential difference.

Based on the fact that common lipid vesicles (liposomes) only cross the pores that are bigger than their own diameter, one would expect negligible aggregate penetration through the openings much smaller than the average vesicle diameter. Figure 1 contains unexpected and unprecedented data that put this expectation in question and require new concepts for explanation.

Results shown in figures 1 and 2 can be interpreted, for example, by generalizing the model of ultradeformable aggregate penetration described by the applicant (see e.g. Crit. Rev. Therapeutic Carrier Syst., 1997). The basic considerations for making such model modification are given in the introductory part of this application.

**Figure 3:** Vesicle transport (penetration) across a microporous barrier, deduced from the delivery of vesicle-associated DPH fluorescence, as function of time. Data suggests that liposomes that are ~3 times bigger than the pores cannot pass these obstacles, in contrast to the comparably large, but much more deformable, mixed lipid vesicles with composition that renders their membranes more flexible.

#### Examples 5-10:

##### Effects of vesicle size and electrical potential difference across the barrier

##### Suspension characteristics:

Total lipid (TL) content 0.5 w-% comprising:

274 mg phosphatidylglycerol (DOPG)

0.1 mol-% DPH (relative to DOPG)

226 mg Tween 80

99 mL phosphate buffer, 10 mM, pH 7

Vesicle/pore size ratio: 5.2

##### Electrical driving force or parameters.

Current: 1.8 mA, 2.3 mA, 2.5 mA, 2.8 mA, 3 mA, 4 mA

Current: density: 0.429 mA/cm<sup>2</sup>, 0.547 mA/cm<sup>2</sup>, 0.595 mA/cm<sup>2</sup>, 0.666 mA/cm<sup>2</sup>,

0.714 mA/cm<sup>2</sup>, 0.952 mA/cm<sup>2</sup>

**Experimental** procedures were as described in examples 1-4. However, in this test series the effect of electrical potential difference was studied. This was first done by using relatively large vesicles which exceeded the average pore size by more than the factor of 5.

**The results** of this experimental series shown in figure 3 document the necessity of

applying at least 1 V potential difference across the barrier. 1.1 V to 1.2 V are sufficient to ensure significant transport of the highly deformable vesicles through 30 nm pores. To transport greater material amount through the barrier, trans-barrier potential in excess of 1.5 V is needed. Electrostatic potential differences of such magnitude then  
 5 results in rather high (opportunistic) electrical currents greater than  $0.5 \text{ mA cm}^{-2}$ .

It is therefore obvious that electro-passage of highly deformable vesicles through a barrier differs qualitatively from the simple electrophoresis or vesicle transport in the bulk. From Ohm's law one would predict that the electrically driven current will  
 10 increase linearly with the transport driving potential, commensurate to the system conductivity / inverse resistance. Such a linear dependence, and constant resistance, is indeed observed during the conventional electrophoresis. In this study, however, a strong nonlinearity was found. This cannot be a consequence of changing barrier properties. The data displayed in figure 3 thus suggest that vesicle capability to cross a  
 15 barrier increases with the applied potential. Similar report was made previously for the hydration-driven transport of highly deformable vesicles across a microporous barrier, and was explained with the mechanosensitivity of highly deformable mixed lipid membranes.

20 **Figure 4:** Temporal characteristics (upper panel) and potential sensitivity (lower panel) of the vesicles with an aggregate/pore size ratio of approximately 5.2, penetrating the transport barrier under influence of an external, transport driving electrical potential.

25

### Examples 11-16:

Suspension characteristics:

As in examples 5-10, except for a decrease in

Vesicle/pore size ratio: 4.6

Electrical driving force or parameters.

Current: 1.4 mA, 1.6 mA, 1.8 mA, 2 mA, 2.5 mA, 3 mA

Current: density: 0.333 mA/cm<sup>2</sup>, 0.381 mA/cm<sup>2</sup>, 0.429 mA/cm<sup>2</sup>, 0.476 mA/cm<sup>2</sup>, 0.595 mA/cm<sup>2</sup>, 0.714 mA/cm<sup>2</sup>

**Experiments** were done and analyzed as discussed in examples 1-11. Notable difference was the decreased relative vesicle size, however, which shifted minimum potential difference required for a substantial vesicle penetration across the barrier to 1.2 V (see figure 4). Even with the highest potential difference studied to date (1.7 V), no clear proof of the saturation of potential-dependent transport increase was obtained. Significant, albeit smaller fluxes were measured with the electrostatic potential differences above 0.8 V, however.

**Figure 5:** Characteristic time course (upper panel) and potential sensitivity (lower panel) of ultradeformable vesicle penetrating through the pores nearly 4.6 narrower than the average aggregate diameter.

**Examples 17-28:**

Suspension characteristics:

Total lipid (TL) content 0.5 w-% comprising:

274 mg phosphatidylglycerol (DOPG)

226 mg Tween 80

0.1 mol-% DPH (relative to DOPG)

99 mL phosphate buffer, 10 mM, pH 7

Vesicle/pore size ratio: 3.5

Electrical driving force or parameters.

Current: 0.4 mA, 0.6 mA, 0.8 mA, 1.2 mA,

1.4 mA, 1.5 mA, 1.6 mA, 2.3 mA, 3 mA,

3.5 mA, 4 mA

Current: density: 0.095 mA/cm<sup>2</sup>, 0.143 mA/cm<sup>2</sup>, 0.190 mA/cm<sup>2</sup>, 0.286 mA/cm<sup>2</sup>, 0.333 mA/cm<sup>2</sup>, 0.357 mA/cm<sup>2</sup>, 0.381 mA/cm<sup>2</sup>, 0.547 mA/cm<sup>2</sup>, 0.714 mA/cm<sup>2</sup>, 0.833 mA/cm<sup>2</sup>, 0.952 mA/cm<sup>2</sup>

**Results.** Proper pore penetration is observed when the transbarrier electrostatic potential difference is at least 1 V. Significant, albeit smaller fluxes are measured when the difference exceeds 0.8 V.

5

Transbarrier potential difference bigger than approximately 1.3 V appears to make the flux of DPH (and by inference, the transport of vesicles) less sensitive to changes in the electrical transbarrier driving potential. It is not entirely clear whether or not the diminished increase in penetration capability, measured with the highest explored potential difference, is diagnostic of saturation of the potential dependent changes in the vesicle transport (see examples 29-35), or else is simply due to the experimental irreproducibility. Data given in the middle panel of figure 5 circumstantially support the former interpretation: if the transport is not analyzed as a function of time but rather as a function of time required to bring certain number of non-confined ions across the barrier, all the curves measured with driving potentials higher than 1 V group closer together.

**Figure 6:** Effect of electrostatic potential difference on the transport of highly deformable, intermediate size vesicles passing 30 nm pores. Upper panel: time course of flux measured under the constant current conditions; middle panel: data as above, but with the time-axis normalized with relatively to the given electrical current; lower panel: capability of ultradeformable vesicles to penetrate pores of fixed-size by electromotion.

25 **Examples 29-35:**

Suspension characteristics:



As in examples 17-28, except for  
Vesicle/pore size ratio: 2.6

Electrical driving force or parameters.

Current: 0.25 mA, 0.4 mA, 1 mA, 1.2 mA, 1.4 mA,  
1.8 mA, 2.3 mA

Current: density: 0.060 mA/cm<sup>2</sup>, 0.095 mA/cm<sup>2</sup>,  
0.238 mA/cm<sup>2</sup>, 0.286 mA/cm<sup>2</sup>,  
0.333 mA/cm<sup>2</sup>, 0.429 mA/cm<sup>2</sup>, 0.548 mA/cm<sup>2</sup>

**Test conditions** in this experimental series were such that the exclusion criterium for the lipid vesicles motion across a barrier with the vesicle/pore size ratio of 2.6 was very weak. (It is known from previously published work by us (Cevc et al., Biochim. Biophys. Acta 1368, 201-215, 1998) and the others that size exclusion begins to govern the transport across microporous barriers when the penetrant/pore size ratio exceeds the value of 2. The flux of vesicle-associated label, consequently, was biphasic in this test series (cf. figure 6). Normalization of the time axis (see the middle panel of figures 5 and 6) does not group the curves together. Rather than this it makes the spread more uniform.

10

Initial slope of material transport curve measured for different transbarrier potential and current values is fairly constant. This is illustrated in lowest panel, which gives normalized slopes of the curves shown in upper panel. The "early part" of measured curves reveals little, if any, voltage dependence. In contrast, the later time flux characteristics (after approx. 1 hour) are indicative of a change in the system properties, which is seen when transbarrier voltage or current is sufficiently high (0.7 V and 0.225 mA cm<sup>-2</sup>, respectively). The observed lag-time is fairly insensitive to the electrical current, as can be seen from the upper panel of figure 2, but does get somewhat shorter with increase in current/potential value.

20

Barrier penetrability can not increase significantly upon changing the applied voltage. It

is therefore more than probable that the above mentioned late penetrability change results from increased capability of lipid aggregates to pass the barrier. We interpret this difference as a sign of a moderately increased vesicle adaptability to pore narrowness. The earlier transbarrier transport, on the other hand, is likely to be due to simple electrophoresis of relatively tiny vesicles. Obviously, many such vesicles are small enough to cross the pores in a barrier, probably in the process of an electrically mediated (or supported) "diffusion".

Penetration capability data illustrated in the lower panel of figure 6 are diagnostic of complete vesicle adaptability (maximum membrane flexibility), as can be seen from the fact that several high potential values are nearly the same.

**Figure 7:** Elektromotion of relatively small, highly deformable vesicles through 30 nm pores in a barrier. Upper panel: absolute ponetration of vesicles, as calculated from the measured DPH flux; middle panel: the same data as above, as a function of normalized time; lower panel: relative penetration capability of tested system (=DPH-derived vesicle flux per unit potential). Two different transport rates ( $\phi_1$  and  $\phi_2$ ) are seen, indicative of two different unerlaying transport phenomena.

### Examples 36-40:

#### Effects of electrolyte concentration

##### Suspension characteristics:

Total lipid (TL) content 0.5 w-% comprising:  
 274 mg phosphatidylglycerol (DOPG)  
 226 mg Tween 80  
 0.1 mol-% DPH (relative to DOPG)  
 Buffer as in previous examples

NaCl concentration (final): 1 mM, 10 mM, 20 mM, 50 mM, 100 mM

Vesicle/pore size ratio: 3.3

Electrical current: 1.2 mA; current density  $0.286 \text{ mA cm}^{-2}$

In this test series we have shown that increasing supporting electrolyte concentration strongly affects the efficiency of electrically driven transport across a microporous barrier. High electrolyte concentrations typically support the salt transport but lower the transbarrier flux of aggregates. Above certain threshold concentration, which is believed  
5 to depend on the barrier as well as penetrant properties, the added salt may bring the transport of large aggregates to a halt.

Comparison of material flux, ion current and driving electrical potential data measured in this and previous set of experiments provides a clue to explaining the salt-dependent  
10 suppression of aggregate electromotion through the narrow pores. The relative contribution of small anions (here  $\text{Cl}^-$ ) flow across the barrier is proportional to the bulk salt concentration. A lower driving potential, consequently, suffices to maintain a constant current across the barrier at higher salt concentrations (see lower panels in figure 7). Lower driving potential simultaneously lessens the ease of, and thus the  
15 probability for, large penetrant adaptability and penetration capability (see upper right panel). The latter is the proviso for an efficient flow of aggregates, however, through the narrow pores. Below certain adaptability value, which is affected by the penetrant/pore size ratio, the deformability of aggregates therefore becomes so low that only insignificant transport takes place.

20

Addition of salt to the suspension of complex aggregates capable of very strong, stress driven deformation therefore detrimentally affects the transbarrier transport.

**Figure 8:** Electrically driven transport of charged, highly deformable vesicles across an artificial barrier with 30 nm pores in the presence of different salt solutions.  
25 Upper left: transbarrier flow of DPH labelled vesicles; upper right: penetration capability of complex aggregates; lower left: electrical potential

that drives the constant current across a barrier as a function of time; lower right: transport driving electrostatic potential as function of bulk NaCl concentration.

5

**Examples 41-50:**

### Changes in the barrier properties and in the test system characteristics

**Suspension characteristics:**

As in examples 17-28

### Electrical driving force or parameters.

As in examples 17-28

Figure 8 shows that the electrical resistance to electrophoretic motion across a barrier increases nearly linearly with the applied electrical potential, but only in certain range. The onset of material flux, in parallel, gets faster (see figure 5 for comparison). This means that the lag-time becomes shorter with increasing transbarrier potential difference. Below the "linear" range, which commences at approximately 1 V for the tested suspension, only insignificant vesicle transport is observed. The tiny flow of aggregate material is then hardly affected by the applied potential or by the changing electrical current.

During experiments done in this test series, pH in the receiver compartment dropped by approximately 1.5 to 1.8 units, nearly independent of the applied voltage. Over the first 20 hour of electrophoresis the change was smaller than 1 unit. (Such a variation was considered to have had only a small, if any, effect on the vesicle electromotion.) In parallel, the donor compartment pH became more alkaline by the corresponding amount. This latter variation did not change the charge on the mixed lipid vesicles, owing to the low  $pK = 2.9$  of ionic PG in the membranes. Lipid degradation, which is 25 faster when the charged membranes diverge from their optimum at  $pH \sim 7.1$ , is believed

to have been insignificant during the first part of experiment at least.

**Figure 9:** Electrically driven transport of charged, highly deformable vesicles across an artificial barrier with 30 nm pores. Upper left: barrier electrical resistance; upper right: electrical potential required to drive constant electrical current across the barrier; lower left: pH value in the receiver compartment containing alginic acid; lower right: pH of suspension of highly flexible vesicles present in the donor compartment.

**Data interpretation.** When the rate of vesicle transport across a barrier is substantial, the electrical potential difference that needs to be applied to drive a constant electrical current through the pores first rapidly decreases and finally increases with time. We believe that the former phenomenon results from the redistribution of highly mobile ions in the system, especially in front of the electrodes and near the barrier. The secondary, and much slower increase, in our opinion, is largely due to the gradual pore clogging by the large or poorly deformable vesicles accumulated in front of the barrier.

Changes on or near the electrodes could partly explain the secondary changes in barrier penetrability / driving potential values. Especially with the high currents we often saw material (alginic acid?) precipitation near the reference electrode. Electrode surface also always turned brown during the course of an experiment; the higher was the applied potential or the bigger was the resulting current the more this was the case. Last but not least, hydrogen and oxygen evolution in the solution, which occasionally led to slight suspension foaming, also could have contributed to the above mentioned barrier resistance changes.

### **Examples 51-53:**

#### Transport characteristics of the skin (epidermis)

Electrical parameters: as shown in figure 9

(Currents are given in the panels)

**Electromotion through the epidermis** in vitro can be used to study some of the characteristics of electrophoresis in vivo. The proviso for this is the use of sufficiently large and intact skin segments with a functional barrier. In order to obtain at least semi-quantitatively reliable data, such skin piece must also be as thin as possible. Ideally, one would like to work with a mere barrier, that is, with the stratum corneum only. In practice it is impossible to achieve this task, owing to the fragility of the horny skin layer. The best that one can do then is to prepare thin but sufficiently extended pieces of the epidermis.

**Electrical resistance of the epidermis** is a good marker for the skin intactness. It is also diagnostic of any major changes in the barrier properties of the organ.

An example for the variable electrical resistance of the skin as a function of time during transepidermal electrophoresis is given in figure 9.

**Figure 10:**Electrical resistance of epidermis during serial electrophoresis experiments done in vitro.

The specific resistance of excised skin (originally somewhat higher than  $10 \text{ kOhm cm}^{-2}$ ) always decreases with the cumulative current that has flown through the skin to approximately 10-20 % of starting value. This observation as well as the starting specific resistance value is comparable to the published information, which gives specific resistance values as below  $20 \text{ kOhm cm}^{-2}$  for human and murine skin. The somewhat higher resistance decrease to approximately 10% of starting value could be due to the difference in total, cumulative current.

We observed no significant difference in electrical resistance, or its time and current variation, between the tested human and porcine skin samples.

**Examples 54-57:**

Barrier (epidermis) thickness effect:

Suspension characteristics:

Total lipid (TL) content 0.5 w-% comprising:

274 mg phosphatidylglycerol (DOPG)

226 mg Tween 80

0.1 mol-% DPH (relative to DOPG)

Buffer as in previous examples

Part A: Vesicle/pore size ratio: 3.3;

electrical current: 0 mA, 1.2 mA (current density 0.286 mA cm<sup>-2</sup>)

electrical potential: difference: 0 V, 2,0 V

Part B: Vesicle/pore size ratio: 2.8

electrical current: 1.2 mA (current density 0.286 mA cm<sup>-2</sup>)

electrical potential: difference: 3.7 V, 5.4 V, and 7 V

5     **Results.** In first experiment (part A), an electrical current of approximately  
0.3 mA cm<sup>-2</sup> was shown to co-transport only a small amount of fluorescently labelled  
ultradeformable vesicles through the thick epidermis, prepared by heat-separation and 2  
hours of trypsin action; only approximately 6 micrograms of material have passed  
through each square centimeter in approx. 4 hours. Then, a collapse in the skin barrier  
10    resulted in strong decrease of electrical resistance of the skin and in a concomitant  
increase of material flow through the (probable) perforated organ.

Further experiments (part B) were done with two skin preparation methods. 2 hours and 7 hours of enzymatic action were used for this purpose, which gave rise to rather thick (5,4 V; 3,7 V) or thin (7 V) specimen, respectively. Moreover, slightly smaller vesicles were used than in part A. This latter difference notwithstanding, the results from repeat experiments have confirmed the trend observed in part A experiment. They also revealed the importance of skin thickness on the effective vesicle flux across the barrier.

After a lag-time of approximately 22 min the transport of ultradeformable vesicles across thin skin, as assessed by means of fluorescent label flux determination in part B, was substantial ( $0.4 \text{ microgramms cm}^{-2} \text{ min}^{-1}$  or approx.  $25 \text{ microgramms cm}^{-2} \text{ h}^{-1}$ , see figure 11). Conversely, an order of magnitude smaller flux was measured with the two thicker epidermis samples. However, even in the case of high flux, saturation was observed. This could result from the clogging of pores in the skin, which are available in limited number, especially under conditions as used in this test.

**Figure 11: A)** Electrically driven transport of ultradeformable vesicles across human epidermis (upper panel), electrical resistance of the barrier (middle panel) and pH in receiver compartment (lower panel) during an electrophoretic experiment.

15      **B) Transport of charged vesicles through thin or thick epidermal samples**  
with an applied electrical potential.  
**Inset** gives the corresponding rate of penetration.

**Example 58:**

**Suspension characteristics:**

**Total lipid (TL) content 0.5 w-% comprising:**

274 mg phosphatidylglycerol (DOPG)

226 mg Tween 80

<sup>3</sup>H-DPPC (relative to DOPG)

Buffer as in previous examples

Vesicle/pore size ratio: 3.5

**Electrical characteristics:**

Electrical current: 0 mA, 0.2 mA

(current density: 0, 0.048 mA cm<sup>-2</sup>)

Electrical potential: difference: 0.5 V

The barrier for this test was prepared by acting with trypsin for 7 hours on a heat-





# CLAIMS

1. A preparation comprising penetrants formed by single molecules or by arrangements of molecules, said penetrants being capable of penetrating the pores of a barrier even when the average diameter of said barrier pores is less than the average diameter of said penetrants, since the penetrants are adaptable to the pores, and said penetrants being capable of transporting agents through said pores, or enabling agent permeation through said pores after the penetrants have entered said pores; the average diameter and the adaptability of said penetrants being selected, and said penetrants and / or said agents being provided with sufficient electrical charges, to enable and / or control agent transport through said pores by said penetrants, or agent permeation through said pores after penetrant entry into said pores, under the influence of a suitable electrical driving force, said selection at the same time maintaining sufficient penetrant stability.

15

2. Preparation according to claim 1, characterized in that said penetrant is provided with sufficient electrical charges, at least when associated with an agent, and the penetrant could, in the absence of an electrical driving force, not readily penetrate the barrier pores; the average diameter, the kind and amount of electrical charges and / or the adaptability of the electrically charged penetrants or the charged associations of penetrant and agent, being selected to achieve, and in case, control said transport through the barrier under the influence of the electrical driving force.

20

3. Preparation according to claim 1, characterized in that said penetrant is provided with sufficient electrical charges, at least when associated with an agent, and the penetrant could penetrate the barrier pores in the absence of an electrical driving force; the average diameter, kind and amount of electrical charges and / or the adaptability of the electrically charged penetrants or the charged associations of penetrant and agent being selected to provide control of the agent transport through the barrier under the influence of an electrical driving force.

25

30

4. Preparation according to any one of claims 1 to 3, said penetrant being capable of penetrating said pores under the influence of a suitable driving force, which may be an electrical driving force when the penetrant is suitably electrically charged, and the agents being sufficient electrically charged to enable and / or control their permeation through the pores of the barrier subsequent to entry of said penetrant into said pores by means of an electrical driving force.

5. Preparation according to any one of claims 1 through 4,  
**characterized in that** the average diameter of the electrically charged penetrants or the  
10 charged association of penetrant and agent, is greater (by at least the factor of 2) than the  
average diameter of the pores of the barrier.

6. Preparation according to any one of claims 1 through 5,  
**characterized in that** the penetrant is formed by an electrically charged single molecule  
15 or an arrangement of electrically charged molecules and is associated with one or  
several charged or uncharged agent molecules.

7. Preparation according to any one of claims 1 through 5,  
**characterized in that** the penetrant is formed by an electrically neutral single molecule  
20 or an arrangement of electrically neutral molecules and is associated with at least one  
electrically charged agent, the quantity of electrical charges being sufficient to enable  
transport.



13. Preparation according to claim 11,  
**characterized in that** the content of the more soluble substance is below 99 %, preferably below 80 % and most preferably below 60 % of the saturation concentration of said substance in the droplet.
- 5
14. Preparation according to any one of claims 8 through 13,  
**characterized in that** the less soluble self-aggregating substance is a lipid-like substance and the more soluble substance is a surfactant.
- 10
15. Preparation according to any one of claims 8 through 14,  
**characterized in that** the average diameter of the penetrant is between 40 nm and 500 nm, preferably between 50 nm and 250 nm, even more preferably between 55 nm and 150 nm and particularly preferably between 60 nm and 120 nm.
- 15
16. Preparation according to any one of claims 8 through 14,  
**characterized in that** the average diameter of the penetrant is 2 to 25 times bigger than the average diameter of the pores in the barrier, preferably between 2.25 and 15 times bigger, even more preferably between 2.5 and 8 times bigger and most preferably between 3 and 6 times bigger than said average pore diameter.
- 20
17. Preparation according to any one of claims 8 through 16,  
**characterized in that** the average net surface charge density on a droplet is between 0.05 Cb m<sup>-2</sup> (Coulomb per square meter) and 0.5 Cb m<sup>-2</sup>, preferably between 0.075 Cb m<sup>-2</sup> and 0.4 Cb m<sup>-2</sup>, and particularly preferably between 0.10 Cb m<sup>-2</sup> and  
25 0.35 Cb m<sup>-2</sup>.
18. Preparation according to any one of claims 8 through 17,  
**characterized in that** the weight amount of droplets in formulations for use on human or animal skin is 0.01 to 40 weight-% of the total preparation mass, in particular  
30 between 0.1 and 30 weight-%, and particularly preferably between 5 and 20 weight-%.

19. Preparation according to any one of claims 8 through 17.

**characterized in that** the weight amount of droplets in formulations for the use on human or animal mucosa is 0,0001 to 30 weight-%.

5           20. Preparation according to any one of claims 8 through 19,  
**characterized in that**, the agent is an adrenocorticostaticum, an adrenolyticum, an androgen or antiandrogen, an antiparasiticum, an anabolicum, an anaestheticum or analgesicum, an analepticum, an antiallergicum, antiarrhythmicum, antiarteroscleroticum, antiasthmaticum and / or bronchospasmolyticum, an  
10   antibioticum, antidrepressivum and / or antipsychoticum, an antidiabeticum, an antidot, antiemeticum, antiepilepticum, antifibrinolyticum, anticonvulsivum or anticholinergicum, an enzyme, coenzyme or a corresponding enzyme inhibitor, an antihistaminicum, antihypertonicum, an antihypotonicum, anticoagulant, antimycoticum, antimyasthenicum, an agent against Morbus Alzheimer or Parkinson, an  
15   antiphlogisticum, antipyreticum, antirheumaticum, antisepticum, a respiratory analepticum or a respiratory stimulant, a broncholyticum, cardi tonicum, chemotherapeuticum, a coronary dilatator, a cytostaticum, a diureticum, a ganglium-blocker, a glucocorticoid, an antinflue agent, a haemostaticum, a hypnoticum, an immunoglobuline or its fragment or any other immunologically active substance such as  
20   an immunomodulator, a cytokine, etc., a bioactive carbohydrate(derivative), a contraceptive, an anti-migraine agent, a corticosteroid, a muscle relaxant, a narcoticum, a neurotherapeutic agent, a (poly)nucleotide, a neurolepticum, a neurotransmitter, a (poly)peptide(derivative), an opiate, an ophthalmicum, a (para)-sympaticomimeticum or (para)sympathicolyticum, a protein(derivative), a psoriasis/neurodermitis drug, a  
25   mydriaticum, a psychostimulant, a rhinologicum, a sleep-inducing agent, a sedating agent, a spasmolyticum, tuberlostaticum, urologicum, a vasoconstrictor or vasodilatator, a virustaticum, a wound-healing substance, an inhibitor (antagonist) or promoter (agonist) for the activity of any of the above-mentioned agents or any combination of such agents.

30

21. Preparation according to any one of claims 8 through 20,  
**characterized in that** the liquid medium characteristics, especially the concentration  
 and the composition of the supporting electrolyte, are selected so as to enable and / or  
 control the rate or the efficiency of transport of the penetrant through the pores of the  
 5 barrier.

22. Preparation according to any one of claims 8 through 21,  
**characterized in that** the supporting electrolyte, in particular a buffer, is selected  
 among monovalent (1 : 1) or other low valency electrolytes, with the bulk concentration  
 10 preferably below 150 mM, more preferably below 100 mM, even more preferably below  
 50 mM, and particularly preferably up to 10 mM.

23. A method for effecting the electrically driven transport of penetrants and  
 associated molecules through the pores in a barrier, as defined in any one of the  
 15 preceding claims,  
**characterized in that** a sufficient electrical potential is applied across the barrier.

24. Method according to claim 23,  
**characterized in that** the electrodes used to generate the electrical potential across the  
 20 barrier are located on opposite sides or on the same side of the barrier and are arranged  
 so as to ensure that most of the resulting electrical current will flow across the barrier.

25. Method according to claims 23 or 24,  
**characterized in that** the applied electrical potential value is chosen to be below 30 V,  
 25 more often below 15 V, and even more preferably below 10 V, per cm<sup>2</sup> of the barrier  
 surface.

26. Method according to claims 23 or 24,  
**characterized in that** the current driven across the barrier by the applied electrical  
 potential is in the physiologically tolerable range, typically below 2 mA cm<sup>-2</sup>,  
 preferably below 1 mA cm<sup>-2</sup>, more preferably below 0.6 mA cm<sup>-2</sup> and most preferably  
 5 up to 0.4 mA cm<sup>-2</sup>.

27. Method according to any one of claims 23 through 26,  
**characterized in that** the electrode size is less than 200 cm<sup>2</sup>, more preferably below  
 100 cm<sup>2</sup>, especially below 50 cm<sup>2</sup>, most preferably below 10 cm<sup>2</sup>, or even below  
 10 5 cm<sup>2</sup>.

28. Method according to any one of claims 23 to 27,  
**characterized in that** the electrically conductive material on or of the electrodes  
 comprises at least one metal, in particular selected from precious metals, such as silver  
 15 or palladium, and / or biocompatible salts or chemical complexes of such metals,  
 preferably the biocompatible chlorides, and most preferably silver chloride.

29. Method according to any one of claims 23 to 28,  
**characterized in that** at least one electrode compartment is loaded with electrically  
 20 charged penetrants.

30. Method according to claim 29,  
**characterized in that** the electrode is loaded at the application site or earlier.

25 31. Method according to claims 29 or 30,  
**characterized in that** the electrode is loaded shortly before application, preferably  
 within 360 minutes, more preferably within 60 minutes and even more preferably within  
 30 minutes.

30 32. Method according to claims 29, 30 or 31,  
**characterized in that** the electrode is loaded with the electrically charged penetrant



pre-associated with molecules to be transported, in particular (biologically active) agents.

33. Method according to claims 29, 30 or 31,  
5 **characterized in that** the electrode is loaded with the penetrant and the molecules to be transported, in particular agents that associate therewith during or after said loading.

34. Method according to any one of claims 23 through 33,  
10 **characterized in that** one or more programmable, preferably small, hand-held or self-supported, for example wrist-watch like, devices for single or repeated use are employed to control the polarity, magnitude and / or time-dependence of applied electric potential.

35. Method according to any one of claims 23 through 34,  
15 **characterized in that** different treatment areas are selected to control the transport.

36. Method according to any one of claims 23 through 35,  
20 **characterized in that** the barrier is pre-treated, before initiating the electrically driven transport of charged penetrants, by a non-occlusive application of suitable penetrants on the modifiable barrier, especially formed by human or animal skin, to increase the number or width of penetratable pores in the barrier subsequently to be used for the electrically driven transport across said pre-treated skin barrier.

37. Method according to claim 36,  
25 **characterized in that** the charged or uncharged penetrants used to pre-treat the barrier are similar or identical with those employed for the subsequent electrically driven transport.

38. Method according to claims 36 or 37,  
30 **characterized in that** the charged or uncharged penetrants are non-occlusively applied for up to 24 hours or even longer, typically for up to 12 hours, especially up to 3 hours, or more preferably for less than 1.5 hours, and in case even for less than 30 min, prior to

the initiation of electrically driven transport of charged penetrants and / or permeants across the barrier.

39. Method according to any one of claims 23 to 38,
- 5 **characterized in that** the transportation rate, i.e. the flux, of charged penetrants through the barrier pores is determined as a function of the applied electrical potential or of the electrical current across the barrier, and the function thus found is then employed to optimize the preparation or application.

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ABSTRACT OF THE DISCLOSURE

It is an object of the invention to provide a preparation comprising penetrants formed by single molecules or by arrangements of molecules, said penetrants being capable of penetrating the pores of a barrier even when the average diameter of said barrier pores is less than the average diameter of said penetrants, since the penetrants are adaptable to the pores, and said penetrants being capable of transporting agents through said pores after the penetrants have entered said pores; the average diameter and the adaptability of said penetrants being selected, and said penetrants and/or said agents being provided with sufficient electrical charges, to enable and/or control agent transport through said pores by said penetrants, or agent permeation through said pores after penetrant entry into said pores, under the influence of a suitable electrical driving force, said selection at the same time maintaining sufficient penetrant stability. It is another object of the invention to provide a method for effecting the electrically driven transport of said penetrants and associates molecules through the pores in a barrier.

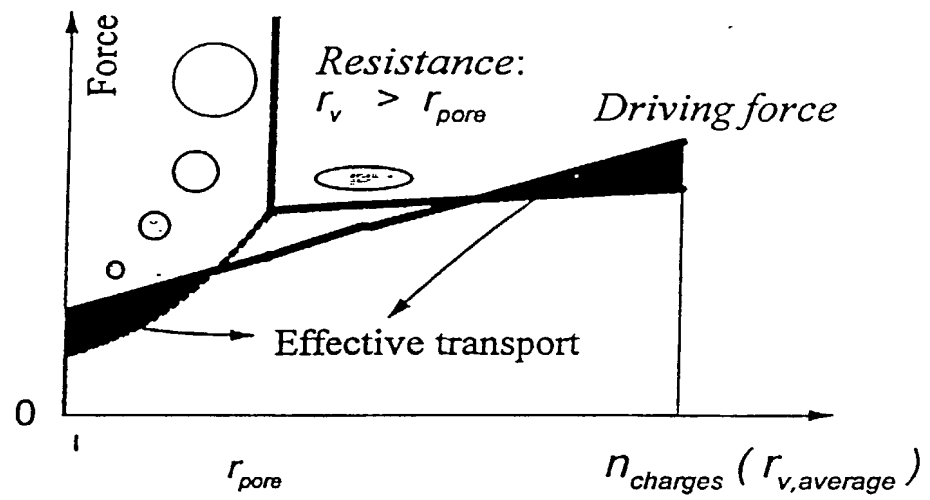


Figure 1

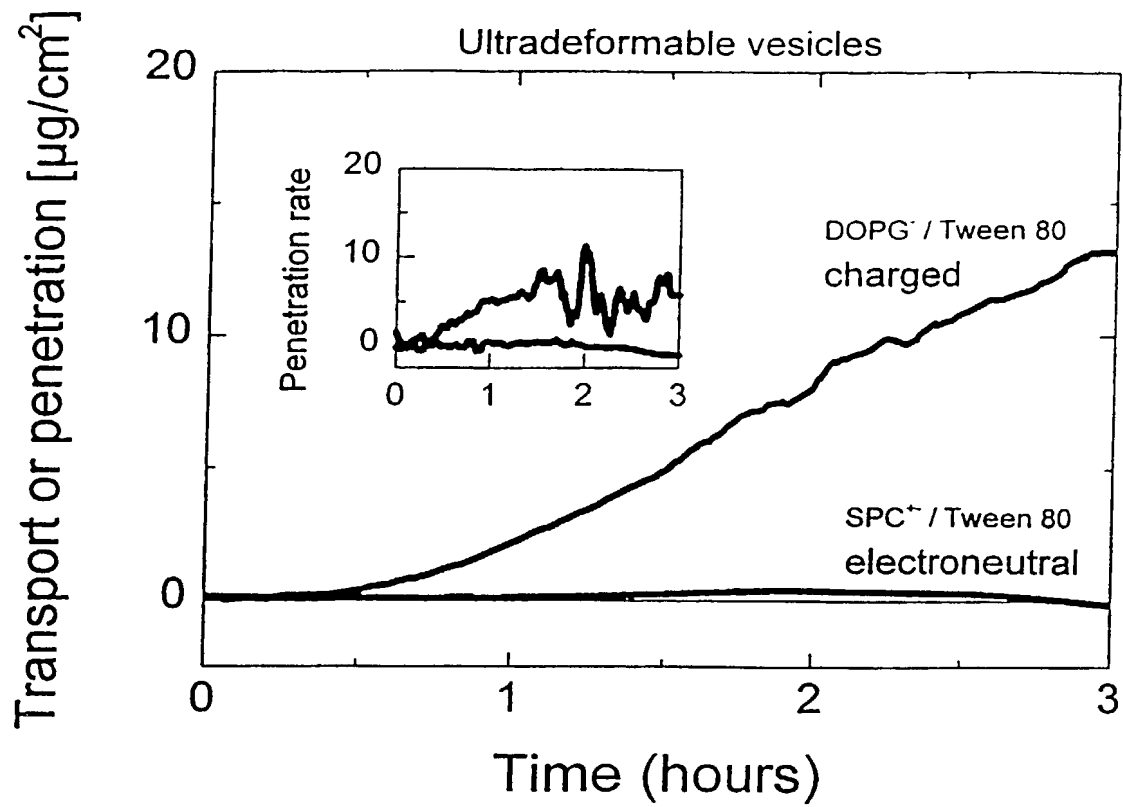


Figure 2

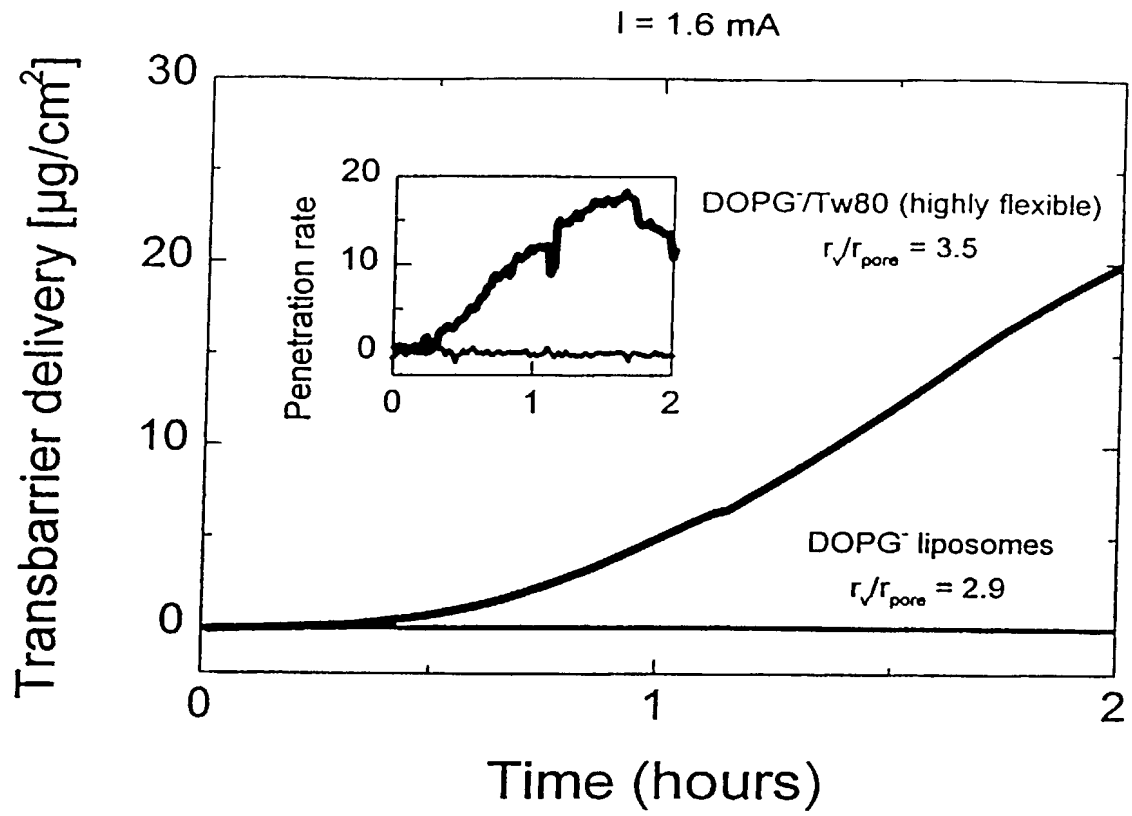


Figure 3

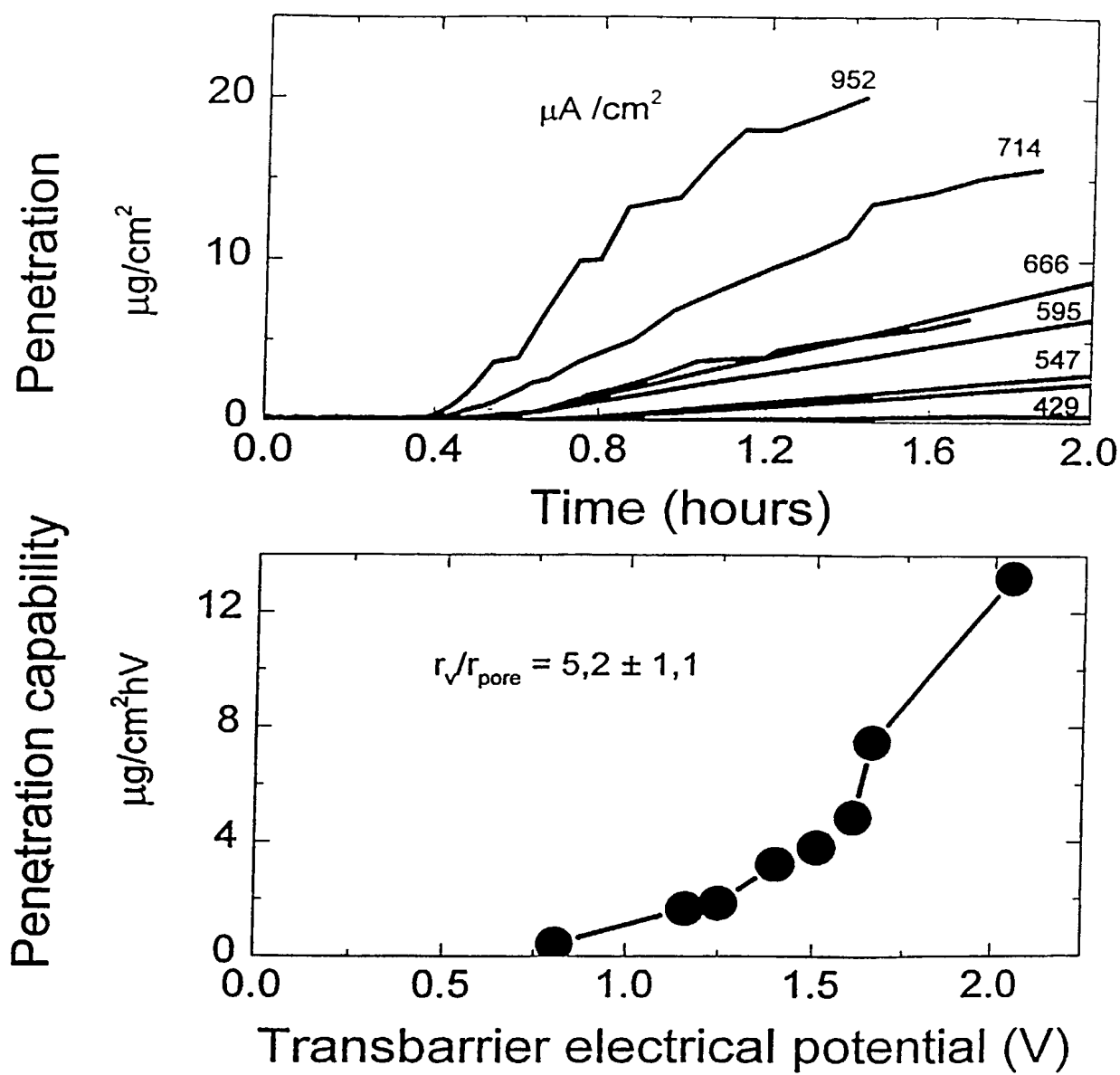


Figure 4

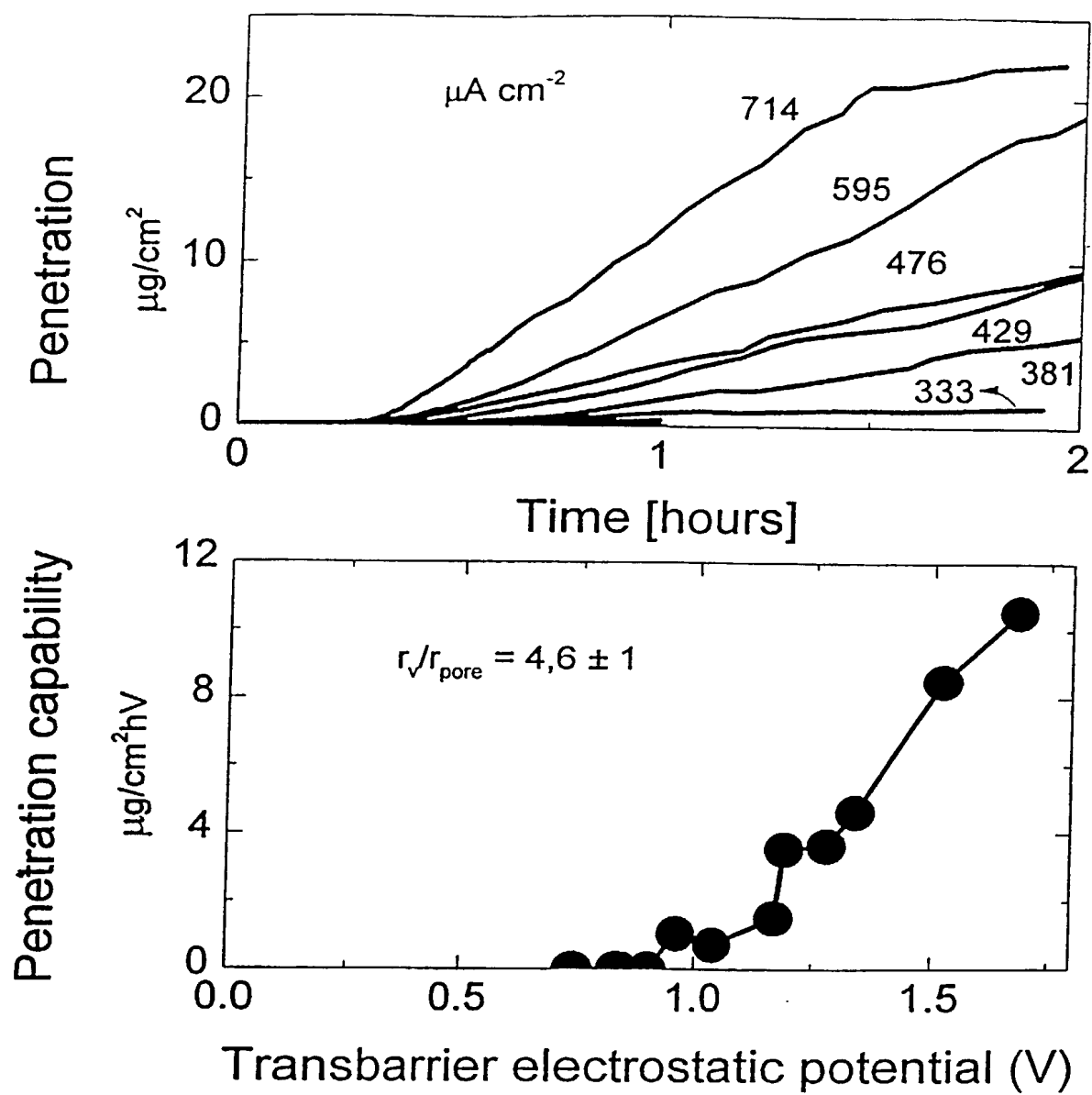


Figure 5



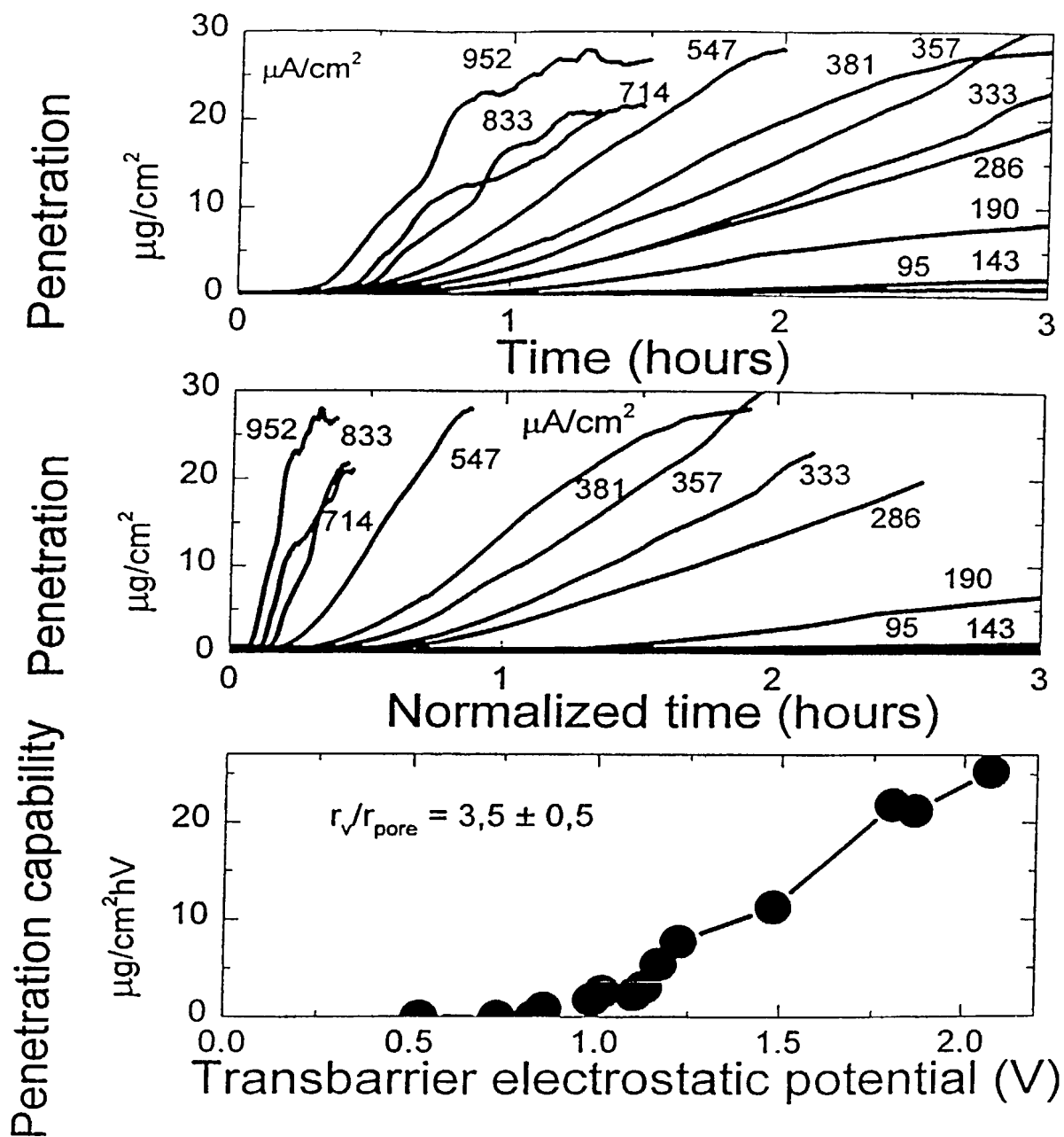


Figure 6

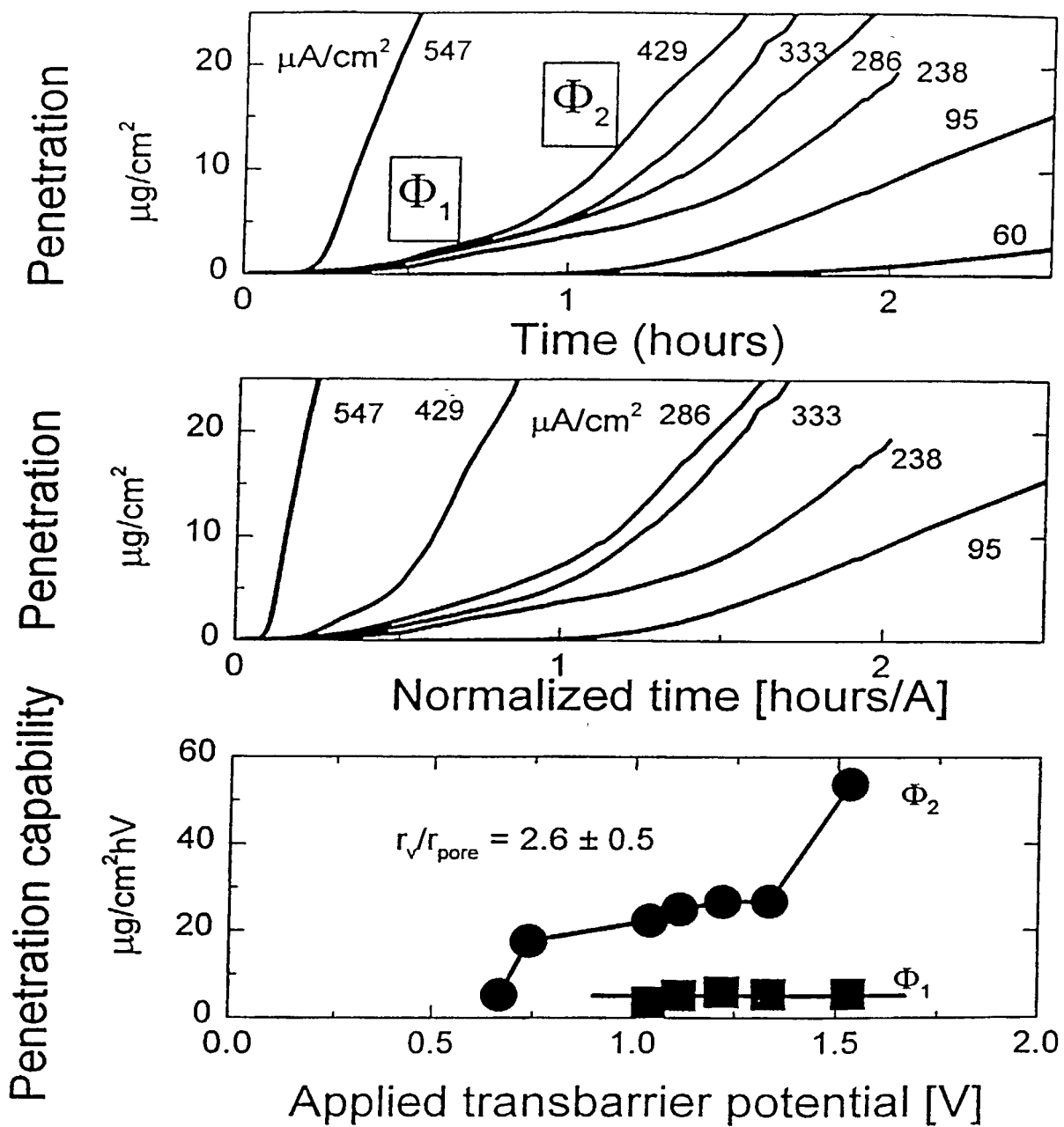


Figure 7

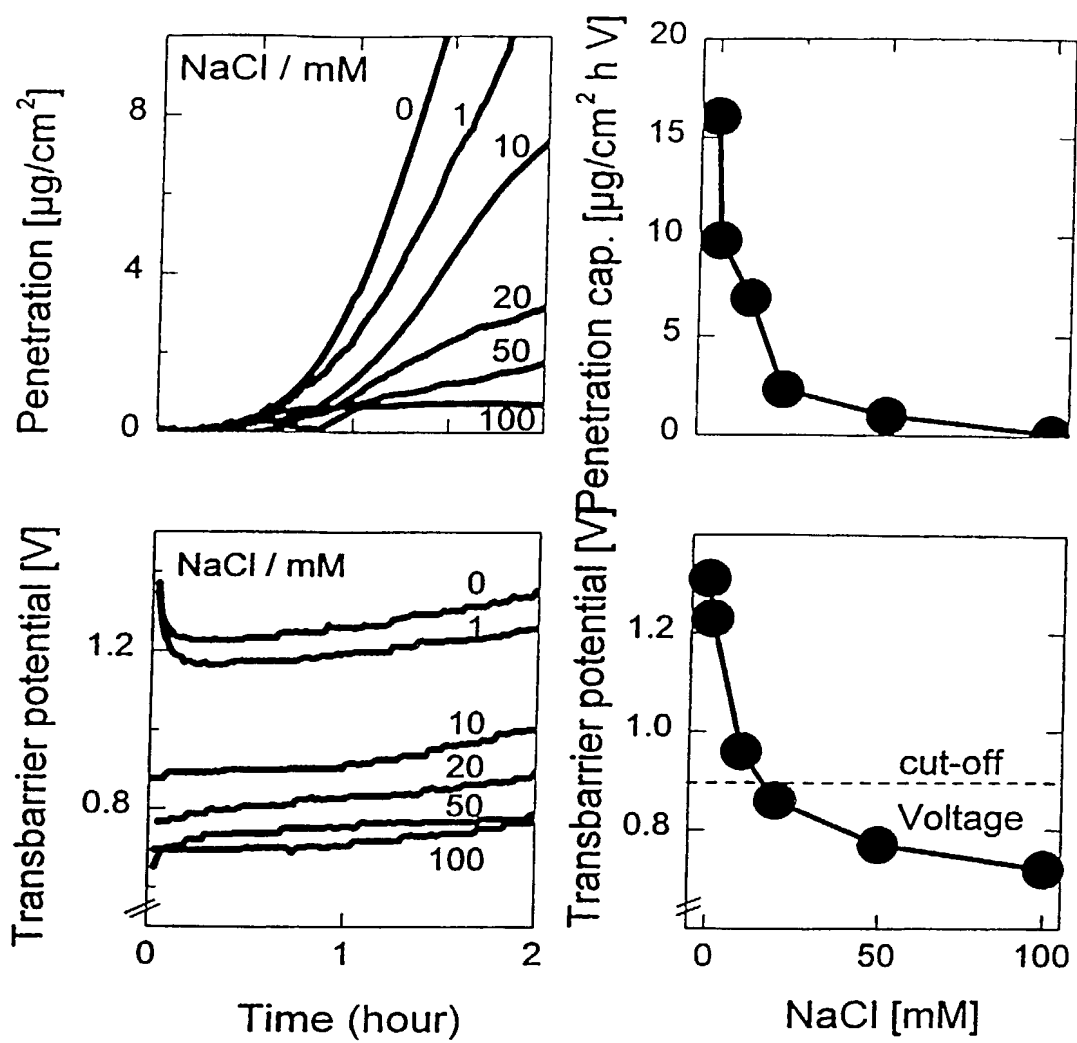


Figure 8

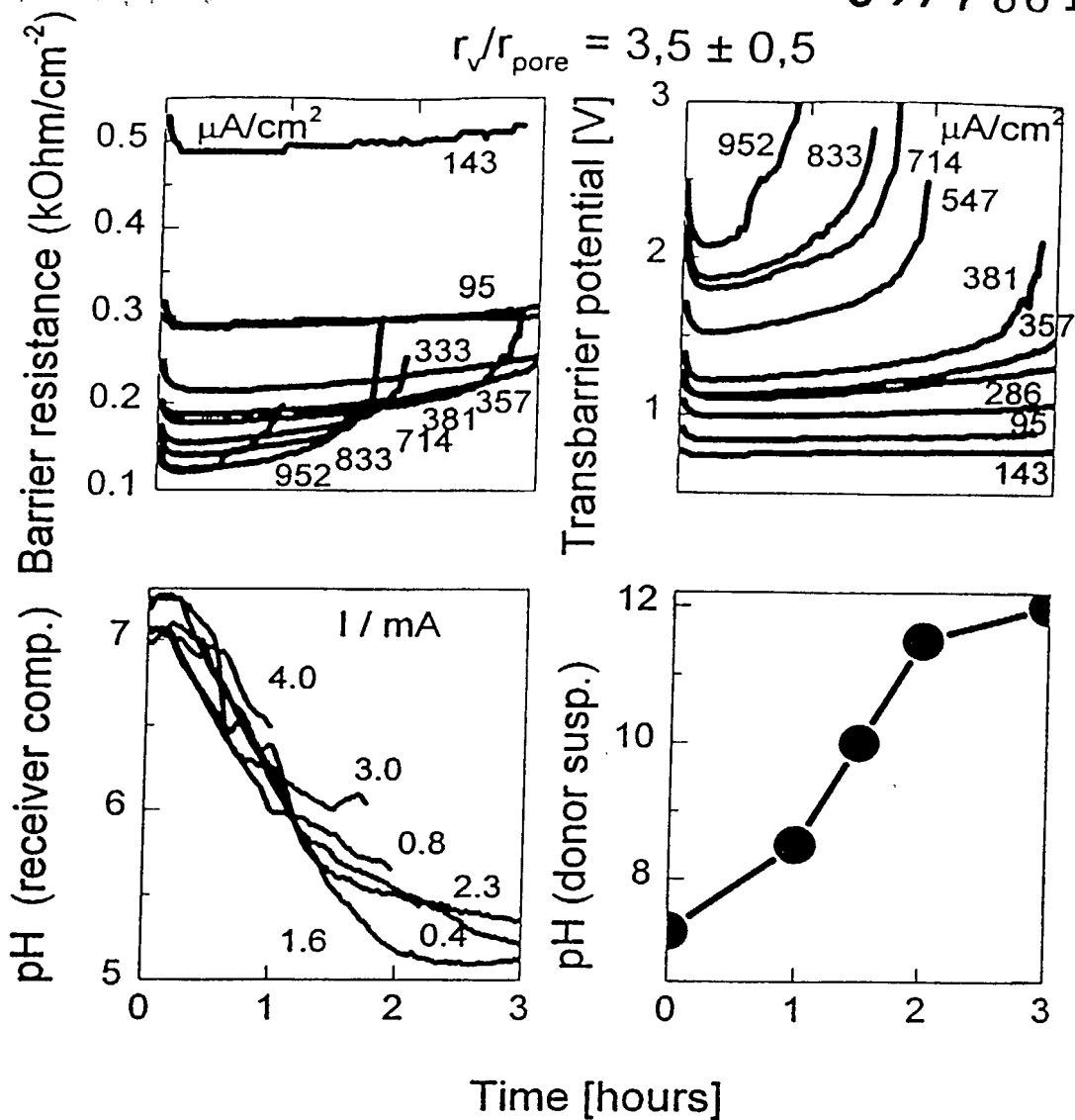


Figure 9

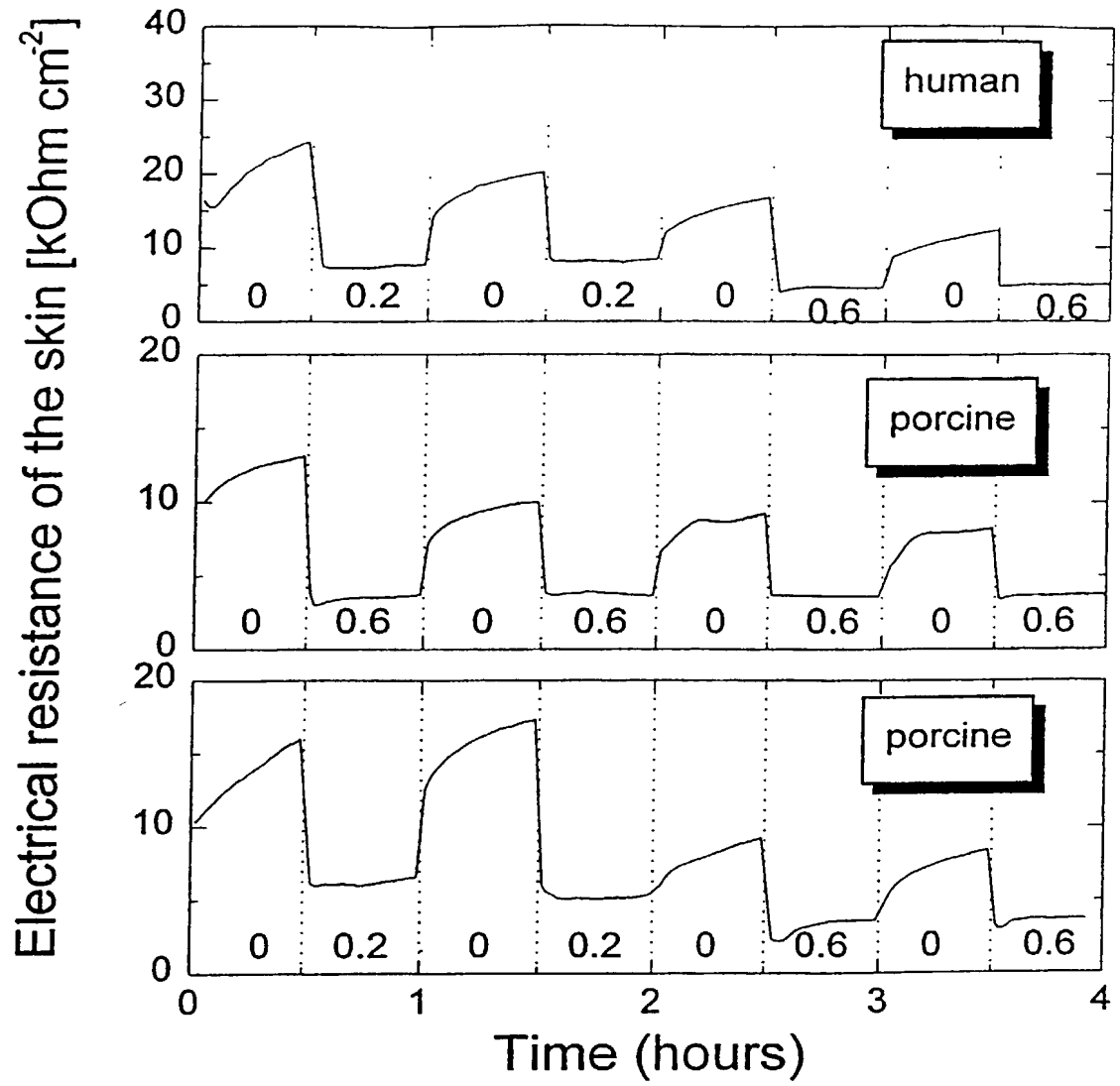


Figure 10

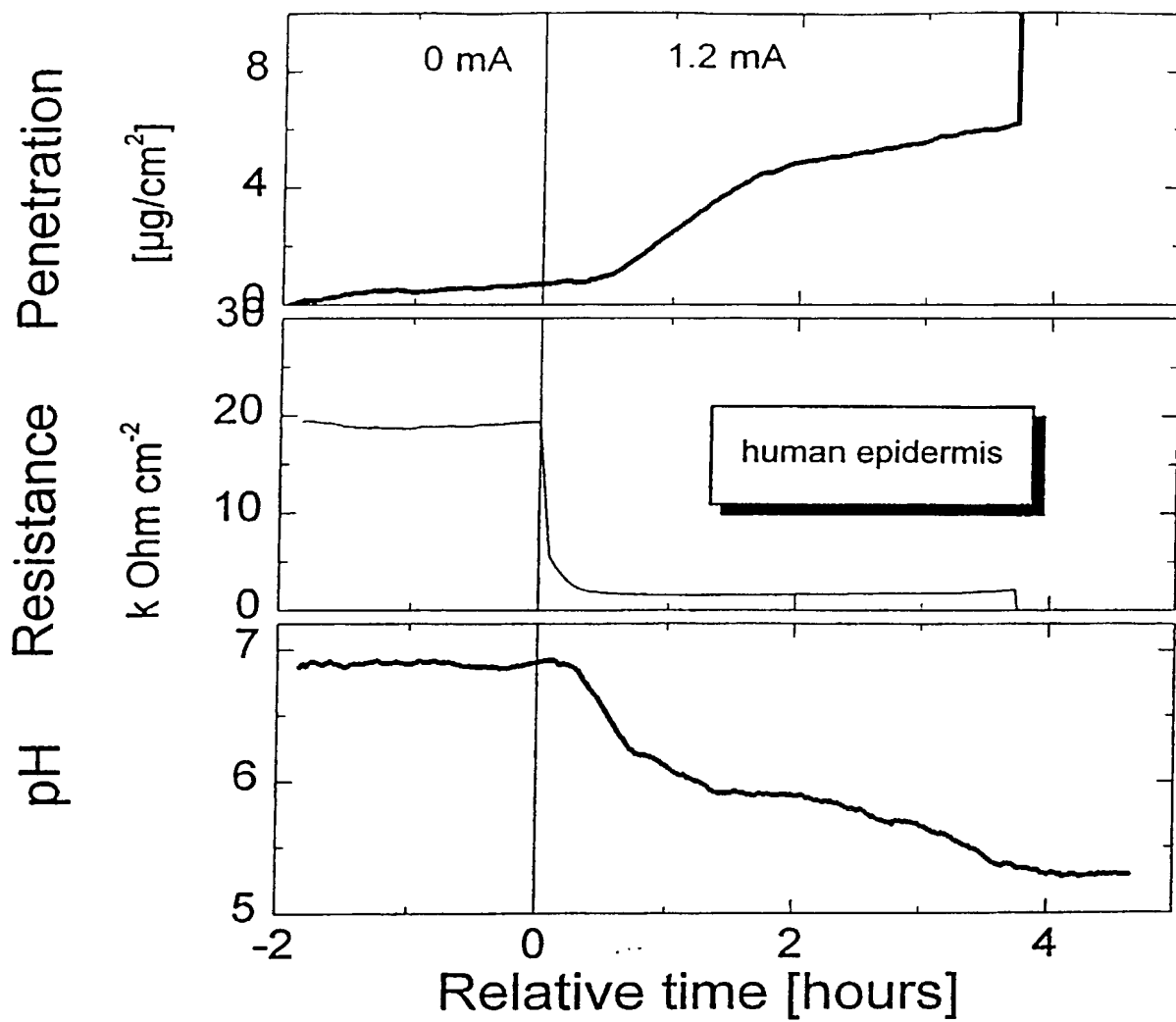


Figure 11A

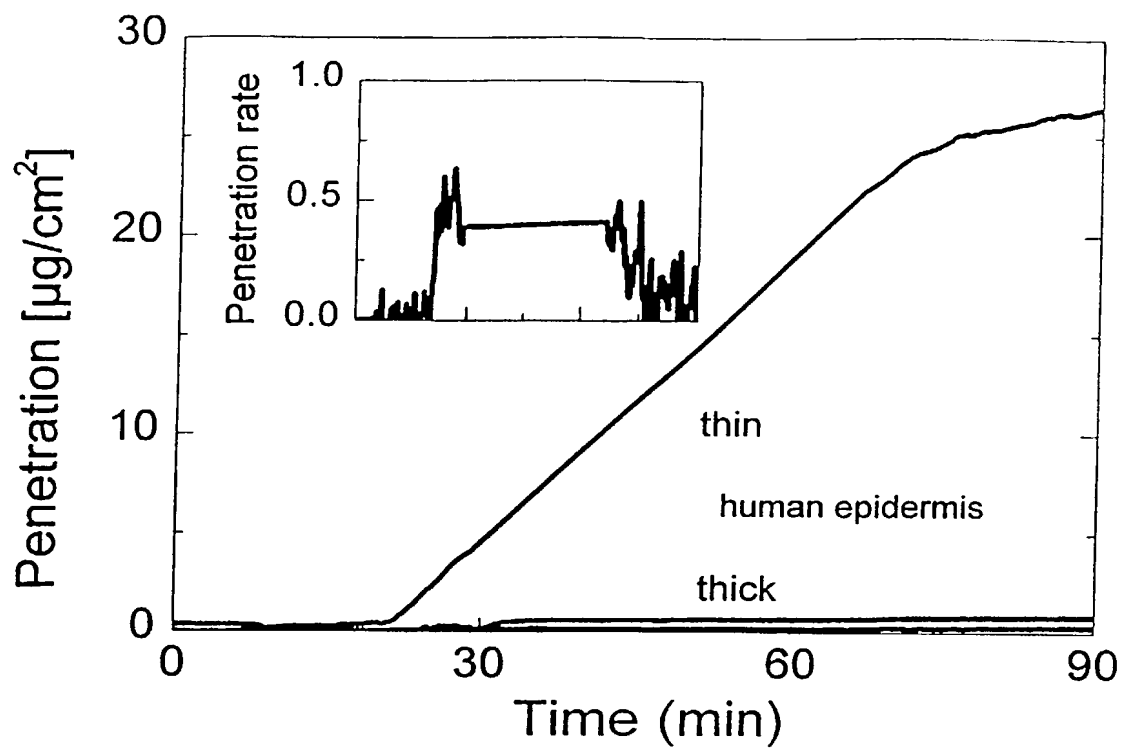


Figure 11B

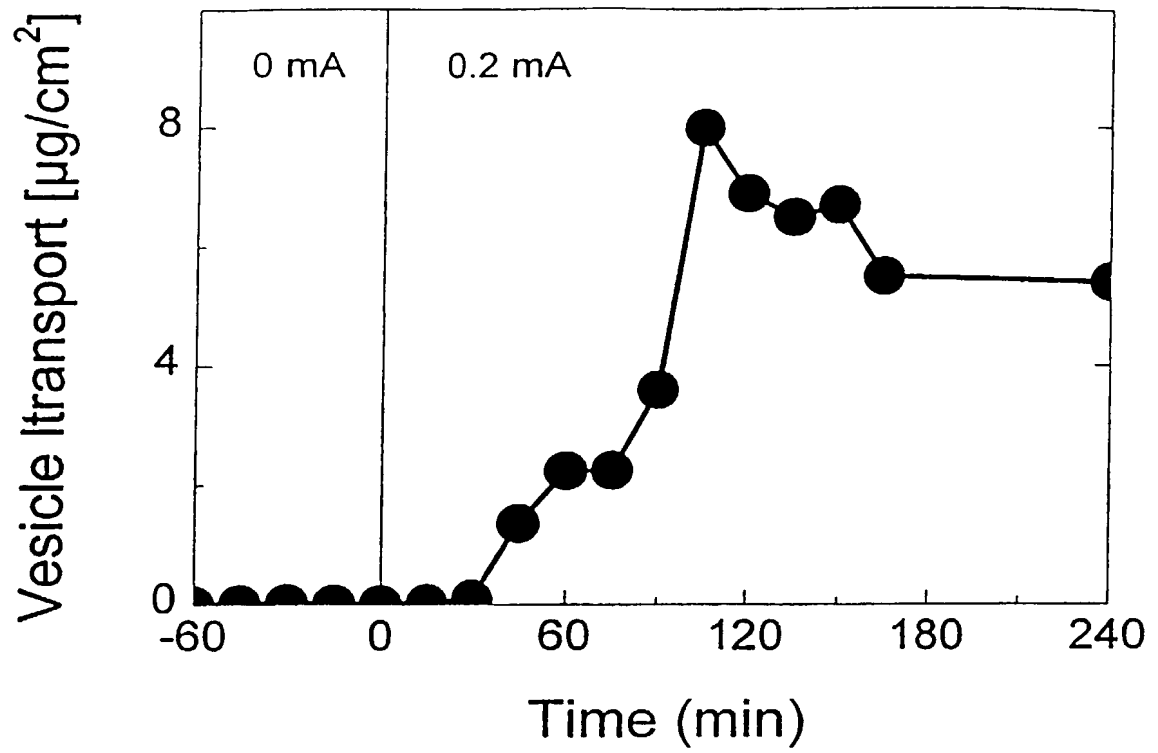
<sup>3</sup>H-DPPC on murine skin

Figure 12



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# **DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)**

☒ Declaration  
Submitted  
with Initial  
Filing

OR

☐ Declaration  
Submitted after Initial  
Filing (surcharge  
(37 CFR 1.16 (e))  
required)

Attorney Docket Number

MPG-108

First Named Inventor

Gregor Cevc

**COMPLETE IF KNOWN**

Application Number

Filing Date

Group Art Unit

Examiner Name

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

**ELECTRICALLY CONTROLLED TRANSPORT OF CHARGED PENETRANTS  
ACROSS BARRIERS**

the specification of which

(Title of the invention)

☒ is attached hereto  
OR

☒ was filed on (MM/DD/YYYY)

as United States Application Number or PCT International

Application Number **PCT/EP98/05539** and was amended on (MM/DD/YYYY) (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 385(b) of any foreign application(s) for patent or inventor's certificate, or 385(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached?	
PCT/EP98/05539	EP	09/01/1998	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	YES <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	NO <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:  
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Application Number(s) Filing Date (MM/DD/YYYY)

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(Page 1 of 2)

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U.S. Parent Application or PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number (if applicable)
PCT/EP98/05539	09/01/98	

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**Name of Sole or First Inventor:** ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle if any)

Family Name or Surname

**GRADOT**

Cevc

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☐ Additional inventors are being named on the \_\_\_\_\_ supplemental Additional Inventor(s) sheet(s) PTO/SP/02A attached hereto